PHARMACOKINETICS, PHARMACOGENETICS AND OPTIMISATION OF TREATMENT WITH NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS IN HIV-INFECTED AFRICAN CHILDREN

By

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Swoją pracę doktorancka dedykuję jedynej osobie, ktora nigdy nie przestala we mnie wierzyc i wspierala mnie przez cale zycie - mojej mamie.

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ABSTRACT

Efavirenz and nevirapine are the most widely used agents for treatment of HIV in children. Sources of variability in their pharmacokinetics and its association with virological outcome and adverse events in African children are poorly characterised, thereby limiting treatment optimisation. To fill this gap we studied population pharmacokinetics (PK) of efavirenz and nevirapine in 478 children from CHAPAS-3 (aged 0.3-1.5 years) using non-linear mixed effects modelling and identified predictors of treatment outcome and PK thresholds most predictive of increased risk of non-suppression using Cox proportional hazards regression models and likelihood profiling.

Efavirenz PK was described by 2-compartment disposition model with 1st-order elimination and transit-compartment absorption and nevirapine by 1-compartment model with elimination through a well-stirred liver model and transit-compartment absorption. Combined effect of SNPs 516GT and 983TC was the strongest predictor of between-subject variability in clearance (89% and 68% decrease between fastest/slowest CYP2B6 metabolic subgroups for efavirenz and nevirapine, respectively). PK was affected by weight, described with allometric scaling. Nevirapine intrinsic clearance displayed diurnal variations (oscillations of amplitude 29%, maximum at 12 noon), while age affected pre-hepatic bioavailability (31.7% lower at birth and increasing exponentially).

In antiretroviral treatment (ART)-naïve children (n=325) increased exposures were associated with decreased risk of non-suppression for both drugs. In efavirenz, risk further increased for children>8 years and for younger boys; in nevirapine for high pre-ART VL, low pre-ART CD4% and low adherence. Thresholds most predictive of non-suppression in efavirenz were: $C_{mid\text{-}dose}$=1.12 mg/L, C_{min}=0.65 mg/L and $AUC_{0\text{-}24}$=28 mg·h/L, while nevirapine had no clear threshold. Adverse events were infrequent in efavirenz, whereas in nevirapine transient transaminase elevations >grade 1 were associated with C_{min}>12.4 mg/L.

Non-nucleoside reverse transcriptase inhibitors dosage guidelines for African children should take into consideration the combined effect of SNPs *CYP2B6* 516G>T and 983T>C, in particular for efavirenz where we observe 10-fold differences in exposures between metabolic subgroups. Moreover the target C_{min} and $AUC_{0\text{-}24}$ could be lowered in children for efavirenz.

Treatment initiation at lower pre-ART VL and higher pre-ART CD4%, increased adherence, and maintaining average C_{min} higher than current target could improve virological suppression of African children treated with nevirapine without increasing toxicity.

CONTRIBUTION TO THE FIELD

FULL LENGTH ORIGINAL ARTICLES

Publication /study 1

A. Bienczak, A. Cook, L. Wiesner, A. Olagunju, V. Mulenga, C. Kityo, A. Kekitiinwa, A. Owen, A. S. Walker, D. M. Gibb, H. McIlleron, D. Burger, and P. Denti, "The impact of genetic polymorphisms on the pharmacokinetics of efavirenz in African children.," *Br. J. Clin. Pharmacol.*, vol. 82, no. 1, pp. 185–98, Mar. 2016.

Publication /study 2

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CONFERENCE PRESENTATIONS

Publication /study 1

"Population Pharmacokinetic Analysis of Efavirenz in African Children using mixture modelling to describe clearance multimodality." Bienczak, A., Fillekes, Q., McIlleron, H., Gibb, D., Burger, D., Denti, P. Jun, 2014 – PAGE, Alicante, Spain (poster)

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DECLARATION

I, Andrzej Bienczak, do hereby declare that this thesis includes four journal papers. All four manuscripts (Chapter 4 -7) have been accepted for print or published in international journals. The contents of each of these manuscripts remains unchanged from that which has been published or submitted for publication. The manuscripts are listed below, with a description of my contribution and the contribution of each author to the study. The original manuscripts in their published form are attached at the end of this thesis.

Chapter 4

Publication /study 1: A. Bienczak, A. Cook, L. Wiesner, A. Olagunju, V. Mulenga, C. Kityo, A. Kekitiinwa, A. Owen, A. S. Walker, D. M. Gibb, H. McIlleron, D. Burger, and P. Denti, "The impact of genetic polymorphisms on the pharmacokinetics of efavirenz in African children.," *Br. J. Clin. Pharmacol.*, vol. 82, no. 1, pp. 185–98, Mar. 2016.

A. Bienczak did the DNA extraction and genotyping of children in CHAPAS-3 at Liverpool University. He conceptualised and concucted the analysis. He was reponsible for merging data from various sources (demographic data from A. Cook, clinical data fom D. Burger and L. Wiesner, self-generated genotypic data and from A. Olagunju), quality control and data cleaning, exploratory analysis, modelling, simulations and interpretation of results, and drafted the manuscript, which was reviewed critically by all authors.

A. Cook was a trial statistitian and data base manager in CHAPAS-3. He maintained the study data base, provided the unformatted data, and critically reviewed the manuscript.

L. Wiesner was responsible for the assaying of the sparse pharmacokinetic samples in CHAPAS-3, and critically reviewed the manuscript.

A. Olagunju genotyped children in ARROW at Liverpool University and critically reviewed the manuscript.

V. Mulenga was a principal investigator in CHAPAS-3. She worked on the study design, acquisition of clinical data, and critically reviewed the manuscript.

C. Kityo was a site principal investigator in CHAPAS-3. He worked on the study design, acquisition of clinical data, and critically reviewed the manuscript.

A. Kekitiinwa was a site principal investigator in CHAPAS-3 and ARROW. She worked on the study design, acquisition of clinical data, and critically reviewed the manuscript.

A. Owen supervised the genotyping, interpretation of PCR assays (both at Liverpool University), and critically reviewed the manuscript.

A. S. Walker was the CHAPAS-3 principal statistitian. She worked on the study design, data interpretation, and critically reviewed the manuscript.

D. M. Gibb was the chief investigator of CHAPAS-3 and a principal investigator in ARROW. She worked on the study design, and critically reviewed the manuscript.

H. McIlleron was the UCT principal investigator. She conceived and worked on the design of the sparse PK and PK-PD components of CHAPAS-3, data interpretation, and critically reviewed the manuscript.

D. Burger worked on the CHAPAS-3 study design and supervised the assaying of intensive pharmacokinetic samples in CHAPAS-3 and ARROW at Radboud University Nijmegen Medical Centre. He worked data interpretation, and critically reviewed the manuscript.

P. Denti supervised the data analysis, interpretation and the writing of the manuscript.

Chapter 5

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A. Bienczak conceptualised and concucted the analysis. He was reponsible for merging data from various sources (demographic data from A. Cook, virological data from L. Wiesner, previosly generated modelling results), quality control and data cleaning, exploratory and main analyses and developed the statistical method for selection of efficacy cut-off. He interpretated the results, and drafted the manuscript, which was reviewed critically by all authors.

P. Denti supervised the data analysis, interpretation and the writing of the manuscript.

A. Cook was a trial statistitian and data base manager in CHAPAS-3. He maintained the study data base, provided the unformatted data, and critically reviewed the manuscript.

L. Wiesner was responsible for the assaying of the sparse pharmacokinetic samples in CHAPAS-3, and critically reviewed the manuscript.

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A. S. Walker was the CHAPAS-3 pricipal statistitian. She worked on the study design, data interpretation, and critically reviewed the manuscript.

H. McIlleron was the UCT principal investigator. She conceived and worked on the design of the sparse PK and PK-PD components of CHAPAS-3, supervised the data analysis, interpretation and the writing of the manuscript.

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H. McIlleron was the UCT principal investigator. She conceived and worked on the design of the sparse PK and PK-PD components of CHAPAS-3, data interpretation, and critically reviewed the manuscript.

P. Denti supervised the data analysis, interpretation and the writing of the manuscript.

Chapter 7

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P. Denti aided the data analysis, interpretation and the writing of the manuscript.

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A. Kekitiinwa was a site principal investigator in CHAPAS-3 and ARROW. She worked on the study design, acquisition of clinical data, and critically reviewed the manuscript.

D. M. Gibb was the chief investigator of CHAPAS-3. She worked on the study design, and critically reviewed the manuscript.

D. Burger worked on the CHAPAS-3 study design and critically reviewed the manuscript.

A. S. Walker was the CHAPAS-3 pricipal statistitian. She worked on the study design, supervised the data analysis, interpretation and the writing of the manuscript.

H. McIlleron was the UCT principal investigator. She conceived and worked on the design of the sparse PK and PK-PD components of CHAPAS-3, supervised the data analysis, interpretation and the writing of the manuscript.

I confirm that no part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree at this or any other university. I hereby grant the University of Cape Town free license to reproduce this thesis in whole or part for the purposes of research or teaching.

I confirm that all authors are aware that these manuscripts were also part of a PhD and have agreed to their use for this purpose.

This thesis is presented for examination in fulfilment of the requirements for the degree of Doctor of Philosophy in Clinical Pharmacology.

Andrzej Bienczak,

Cape Town, 14th of Dec, 2017

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

3TC – lamivudine

ABC – abacavir

AIC – Akaike Information Criterion

AIDS - Acquired Immunodeficiency Syndrome

ALT - Alanine aminotransferase

APV - amprenavir

ARROW - Antiretroviral Research for Watoto

ART – anti-retroviral treatment

ARV – anti-retroviral

AST - Aspartate aminotransferase

ATV – atazanavir

AUC – area under the curve

BD – twice a day

BOV – between-occasion variability

BSV – between-subject variability

BSA – body surface area

CD4 – helper T lymphocyte

CHAPAS - Children with HIV in Africa – Pharmacokinetics and Adherence of Simple Antiretroviral Regimens

C12h – concentration 12h post dose

C24h – concentration 24h post dose

CL – clearance

C_{max} – maximum drug concentration

CMD - clinically driven monitoring

C_{min} – trough drug concentration

CNS – central nervous system

CYP450 – cytochrome 450

d4T - stavudine

ddI – didanosine

DNA - deoxyribonucleic acid

DRV - darunavir

DTG – dolutegravir

EMA – European Medicines Agency

EBE – Empirical Bayes Estimate

EFV – efavirenz

ENF – efuvirtide

ETR – etravirine

EVG(/c) – elvitegravir (boosted with corbicistat)

F1 – bioavailability

fAPV – fosamprenavir

FDA – Food and Drug Administration

FDC – fixed dose combination

FTC - emtricitabine

GOF – goodness of fit

HIV – human immunodeficiency virus

ICH – International Conference on Harmonisation

ka - absorption rate constant

LCM - laboratory plus clinical monitoring

LPV - lopinavir

MRC - maraviroc

NVP – nevirapine

OFV – objective function value

PD – pharmacodynamics or pharmacodynamic

P-gp - P-glycoprotein

PGx - pharmacogenetics

PK – pharmacokinetics or pharmacokinetic

POP-PK – population pharmacokinetics

QD – once a day (or daily)

pMTCT – prevention of mother to child transmission

RAL – raltegravir

RNA - ribonucleic acid

RPV - rilprivirine

RUV – residual unexplained variability

RT – reverse transcriptase

$t_{1/2}$ – half life

TAF – tenofovir alafenamide

TB - tuberculosis

TDF - tenofovir disoproxil fumarate

TDM – therapeutic drug monitoring

TPV – tipranavir

UGT - uridine 5'-diphospho-glucuronosyltransferase

V – volume of distribution

V_{cen} – volume of central compartment

V_{per} – volume of peripheral compartment

WHO – World Health Organisation

ZDV – zidovudine

/r – ritonavir PK boosting

CHAPTER 1: INTRODUCTION

1.1 Context

Without any doubt, acquired immunodeficiency syndrome (AIDS) caused by the infection with human immunodeficiency virus (HIV) is one of the world's most serious health challenges.[1,2] Over the years, HIV infection changed its course from being a certain death sentence, reflected in a 1-year AIDS mortality of 51% in 1986, to merely a chronic disease nowadays.[3,4] This was possible as a result of advances in understanding the biology and pathogenesis of the virus, leading to numerous new drugs developed for treatment of HIV infection. A major breakthrough was the introduction of combination treatment consisting of 3 or more drugs from 2 or more different classes,[5] which dramatically improved the success of treatment. A more recent milestone was the change in treatment guidelines recommending early initiation of ART in all newly diagnosed HIV-infected individuals, regardless of CD4 cell count.[6] Nonetheless, by the end of 2015 there were 36.7 million people worldwide living with HIV with over half of them living in the eastern and southern Africa, where the incidence of new infections is the highest.[7,8] In 2014 3.2 million of children were globally living with HIV and over 90% of them lived in sub-Saharan Africa.[9] According to the World Health Organisation (WHO) the actual number of children in Africa living with HIV might be higher than most reported statistics[10] and an effective and optimised treatment for children should be a priority.

The success of the HIV treatment depends on a number of factors starting with a timely diagnosis and start of the therapy, through availability of effective therapeutics and appropriate formulations, drug supply, and lastly validated treatment guidelines advising on the most optimal dosage of prescribed drugs. In the last years, treatment formularies for HIV-infected adults changed substantially through introduction of newer antiretroviral (ARV) agents (such as integrase inhibitors and new generation non-nucleoside reverse transcriptase inhibitors [NNRTIs]) which provide reduced number of side effects and superior efficacy, and are available as all-in-one fixed dose combination (FDC) tablets.[6] At the same time, treatment options for HIV-infected children are based on agents no longer recommended as first choice in adults and further limited by lack of age appropriate formulations. In its 2010 recommendations, WHO highlighted a number of shortcomings of paediatric antiretroviral formulations: reduced shelf-life, high alcohol content, poor palatability, and storage difficulties (especially in resource limited setting).[11] It has been speculated that increased time to viral suppression and higher mortality rates, especially among younger children starting ART, could be attributed to poor taste of paediatric liquid formulations causing insufficient compliance.[12] In addition, a widespread approach of treating children with crushed adult tablets or sprinkled capsule content could have an unpredictable effect on the pharmacokinetics (PK) of the drugs and in consequence significantly contribute towards treatment failure.[13,14] Lastly, until recently, it was widely

acceptable to derive the paediatric dose by linear scaling of the adult dose based on weight, which led to various cases of treatment with sub therapeutic dose or overdosing in children.[15–18]

The lack of availability of paediatric formulations and absence of clinical testing in children was acknowledged by the International Conference on Harmonisation (ICH), Food and Drug Administration (FDA), and European Medicines Agency (EMA) as a major safety concern leading to introduction of new paediatric regulations in the USA and the EU.[19–24] The new regulations encouraged paediatric drug development and drug testing in children on both sides of the Atlantic.[25–28] Unfortunately, similar regulations have not been approved in any of the African countries, nor did they incentivise development of paediatric formulations for established treatments available as generic counterparts. The results of trials conducted in children in the US or the European countries cannot be directly applied in the African population without prior validation. Numerous publications show that the prevalence of various genetic polymorphisms determining the activity of a number of metabolic pathways involved in biotransformation of various drugs is different between Caucasians and black Africans.[29–45] This can lead to differences in systemic drug exposure, which can have an impact on treatment efficacy and safety. Differences in pharmacokinetics can be further explained through a number of environmental factors, e.g. malnutrition,[14,46,47] as well as different prevalence of co-infections and associated concomitant medications.[48–52] Lastly, treatment of African children is often conducted in a resource limited setting where the recommended first line options and their formulations might be different to the drugs tested and used in the developed countries.

In order to assist international organisations and governments access to generic antiretroviral drugs of acceptable quality and development of age appropriate paediatric formulations, WHO started a number of initiatives, the first one being the 2001 prequalification program[53] (in collaboration with UN agencies), followed by two technical documents in 2004[54] and 2005[55] focused solely on children. This stimulated development of the first paediatric 3-in-1 solid dispersible FCD containing NNRTI nevirapine (NVP) and 2 nucleoside reverse transcriptase inhibitors (NRTIs) stavudine and lamivudine (d4T and 3TC) and a number of similar 2-in-1 formulations containing combination of 2 NRTI backbone drugs recommended in children – abacavir (ABC), d4T, or zidovudine (ZDV), combined with 3TC. The tablets were developed to facilitate paediatric ARV weight band dosing suggested in 2004 UNICEF/WHO Technical Consultation[54] and incorporated in 2006 WHO guidelines.[56] More recently, following the change in treatment guidelines, Cipla India developed a new paediatric double scored efavirenz (EFV) 600 mg tablet.

The most recent WHO guidelines[6] endorse NNRTI efavirenz combined with two NRTIs (most commonly ABC and 3TC) for treatment of HIV in children >3 years old. First line treatment in children <3 years should consist of a combination of 2 NRTIs with ritonavir-boosted lopinavir (LPV/r), but nevirapine-based ART is listed as an alternative regimen.[6] Only combinations of the aforementioned NRTIs (ABC, d4T, or ZDV with 3TC) and nevirapine are currently available as paediatric solid FDC tablets. Their introduction significantly reduced the cost of ART in children and increased treatment feasibility, making them the most widely used options for treatment of paediatric HIV in resource-limited setting and improving its coverage in this vulnerable population. Despite some concerns how exposure to nevirapine for prevention of mother to child transmission of HIV virus (pMTCT) might affect its efficacy in later life[57] recent reports confirm it is not compromised[58–62] and its use in children persists.

Despite proven efficacy and acceptable tolerability of efavirenz- and nevirapine-based ART, both drugs exhibit high levels of variably in individual exposures. This variability in adults was attributed largely to single nucleotide polymorphisms (SNPs) in the *CYP2B6* gene which encodes the key metabolising enzyme for both drugs.[43,63–65] A number of SNPs related to functionality of this CYP450 metabolic pathway have been studied and documented and it has been shown that the prevalence of those polymorphisms varies greatly between Caucasian and black African populations.[29,34–36,39,66–68] The key identified *CYP2B6* polymorphisms are: 516G>T (rs3745274),[69] 983T>C (rs28399499),[43] and 15582C>T (rs4803419)[70]. These SNPs have been reasonably well studied in adults, leading to identification of four distinct metaboliser subcategories,[71] nonetheless the number of similar investigations in children is limited.[43,72,73] Until recently, the paediatric investigations into their effect were restricted to 516G>T,[74,75] and even though a recent study in South African adults and children described the association between SNP 983T>C and efavirenz concentrations,[76] the combined effect of both those SNPs was not quantified. The drug exposures obtained under the simplified WHO weight band dosing with use of the new paediatric nevirapine FDC tablets were evaluated in African children in CHAPAS-1[33,37,77] and in ARROW[41] for efavirenz but no prior investigation evaluated the combined effect of 516G>T|983T>C SNP-vector on exposures across weight bands for neither of the NRTIs.

Cytochrome 450 (CYP450) enzymes activity is regulated by a number of nuclear receptors. Nuclear receptors are ligand-activated transcription factors that control various cell and organism functions on a molecular level by up- or down-regulating expression of proteins.[78] CYP450 activity is influenced mostly by pregnane (steroid) X receptor (PXR) and the constitutive androstane receptor (CAR). [78–80] As a consequence, on top of SNPs in genes coding CYP450 enzymes, also polymorphisms in genes coding these receptors (*NR1|2* for PXR and *NR1|3* for CAR) can indirectly impact drug concentrations.[81,82] Publications show that the polymorphisms in *NR1|2* and *NR1|3* have different frequencies in various

ethnic groups. [83,84] Their effect has been evaluated to date in only one study in efavirenz in African children[76] and never been previously studied in nevirapine.

The metabolism of nevirapine is mediated to a large extent also through CYP3A4 pathway[65] whose pharmacogenetics is less studied. Even though not confirmed for nevirapine, systemic exposures of CYP3A substrates have been shown to be altered by SNPs rs35599367 (*CYP3A4**22)[85,86] and rs776746 (*CYP3A5**1).[87,88] Furthermore, CYP3A activity exhibits diurnal variation with clearance rates increased during the day and reduced at night.[89,90] Differences between morning and evening nevirapine trough concentrations (C_{min}) have been previously reported [91] and may relate to diurnal variation in CYP3A-mediated effects on its clearance. In addition nevirapine PK is speculated to be affected by polymorphisms of *ABCC10*, a gene coding an efflux transporter.[92] Information on the outlined effects in children is either scarce or absent.

The fundamental paradigm of pharmacology is a relationship between drug concentrations (or PK) and its effect (or pharmacodynamics - PD).[93] It is the drug at the site of action that drives the observed effect. In order to achieve optimal treatment effects the drug concentrations should be maintained within certain level, usually described by its therapeutic range. Insufficient amounts of a drug can be the cause of inadequate treatment effect, or in case of antiretrovirals development of drug resistance. On contrary excessive amounts cause unwanted side effects.

For efavirenz concentration rage of 1 – 4 mg/L has been widely accepted as optimal.[94,95] Suboptimal efavirenz exposures have been associated with increased risk of treatment failure and high concentrations with central nervous system (CNS) side-effects.[69,94] A number of studies showed that the efavirenz adverse event profile in adults differs from children, indicating lower incidence of the unwanted effects in the latter.[96–99] The current therapeutic range for efavirenz was derived in a study in adults, mostly of European descent, and was based on a random drug concentration measured 14.0 +/- 2.7 h after dose intake,[94] but is customarily applied to mid-dose as well as trough exposures in all populations. A few paediatric investigations evaluated the efficacy threshold for efavirenz,[32,96,100,101] but the majority of them was limited to evaluation of pre-defined cut-offs based on exposure distribution in the tested population.[32,96,100] In addition the results of ENCORE1,[102,103] showing that the standard 600 mg efavirenz dose in adults can be reduced to 400 mg daily without loss of efficacy, have prompted discussions on the validity of the widely accepted efficacy threshold of >1 mg/L and justify a new investigation into the most optimal concentration range in children.

Based on the concentration-response relationship, a therapeutic range of 3–8 mg/L has been suggested for nevirapine therapeutic drug monitoring (TDM).[95] However, several studies failed to

confirm these associations[72,104,105] and low incidences of nevirapine-related adverse events (AEs) have been reported in low-income settings[106] and in African children.[107,108] Despite widespread use, few studies have investigated the PK determinants of efficacy of nevirapine-based regimens in children. The predictive power of the suggested targets has also never been thoroughly investigated in black Africans or in children. Whether these pharmacokinetic targets should be universally applied across populations was recently questioned.[72]

To bridge the existing gap in knowledge and gain clinical proof of efficacy and safety of the treatment with nevirapine FDC tablets and new paediatric double scored efavirenz 600mg tablets in black African children, a multinational clinical trial was commissioned (CHAPAS-3 – Children with HIV in Africa – Pharmacokinetics and Acceptability/Adherence of Simple Antiretroviral Regimens). Presented thesis is the first analysis of pharmacokinetic and clinical data for efavirenz and nevirapine from this study, which were enriched with previously analysed data from trials CHAPAS-1 and ARROW. The current state of knowledge being the context for this thesis is discussed in more detail in Chapter 2.

1.2 Hypothesis:

We hypothesise that single nucleotide polymorphisms in *CYP2B6* are the main predictors of variability in efavirenz and nevirapine concentrations in HIV-infected African children treated with paediatric solid FDC tablets, and that dose adjustment based on individual genotype alone could provide an optimal treatment outcome.

1.3 Key research questions:

1. Is the inter-individual variability in efavirenz and nevirapine concentrations in African children predicted by single nucleotide polymorphisms in *CYP2B6* metabolic pathway alone, or in combination with selected polymorphisms in additional genes (*CYP3A4*, *CYPCA5*, *ABCB1*, *NR1*|*2, NR1*|*3*), or other demographic characteristics? (**Publication/study 1** and **Publication/study 3**)

2. Do average efavirenz and nevirapine exposures differ between WHO 2010 dosing weight bands and different metabolic subgroups, determined by individual *CYP2B6* genotype, and could genotype based dose adjustment provide more balanced exposures between metabolic subgroups in African children? (**Publication/study 1** and **Publication/study 3**)

3. Are efavirenz and nevirapine concentrations predictive of virological response and adverse events in African children treaded with paediatric solid FDC formulations, and what is the contribution of other variables to the observed treatment effects? (**Publication/study 2** and **Publication/study 4**)

4. What thresholds in efavirenz and nevirapine concentrations are most predictive of virological suppression and adverse events in African children? (**Publication/study 2** and **Publication/study 4**)

1.4 Outline of thesis

Chapter 2 provides background information on HIV in terms of its epidemiology, disease pathogenesis and treatment. It outlines the current guidelines for treatment of HIV-infected children. It describes how the knowledge of pharmacokinetic (PK) and pharmacodynamic (PD) properties of a drug is paramount for treatment optimisation. It summarizes the efavirenz and nevirapine PK and PD, including pharmacogenetics, predictors of virological response and safety, with particular focus on the paediatric studies into PK and determinants of treatment response. It also gives summary of the published population PK models for both drugs.

Chapter 3 presents details of the clinical studies included in this thesis (study design, objectives, population and inclusion/exclusion criteria, chronology of the studies) and elaborates on the statistical methods employed with justification of their choice.

Chapter 4 (Publication/study 1) presents a pharmacokinetic study of efavirenz in African children treated with paediatric solid FDC tablets. The analysis is conducted on data from CHAPAS-3 enriched with PK sub-study of ARROW. The data is analysed using non-linear mixed effects modelling. The main predictors of efavirenz PK are the combined effect of SNP 516T>T and 983T>C and size (weight). Pharmacokinetic simulations are implemented on the final model to aid genotype based dose optimisation and a new dosing algorithm was suggested. The efavirenz pharmacokinetic/pharmacogenetic study is included as a full manuscript with the abstract, introduction, methods, results and discussion and its content has not been modified.

Chapter 5 (Publication/study 2) presents a pharmacokinetic/pharmacodynamic study of efavirenz in African children treated with paediatric solid FDC tablets. The analysis is limited to data from CHAPAS-3. An exploratory analysis is performed using descriptive statistics and a selection of statistical tests, while the main analysis is performed using Cox proportional hazards regression. The study presents a new statistical method for identification of efavirenz concentration threshold most predictive of increased risk of virological non-suppression based on likelihood profiling, describes the concentration-response relationship, and identifies predictors of virological response. The efavirenz pharmacokinetic/pharmacodynamic study is included as a full manuscript with the abstract, introduction, methods, results and discussion and its content has not been modified.

Chapter 6 (Publication/study 3) presents pharmacokinetic study of nevirapine in African children treated with paediatric solid FDC tablets. The analysis is conducted on data from CHAPAS-3 enriched

with PK sub-study of CHAPAS-1. Nevirapine PK is described using a semi-mechanistic population pharmacokinetic model with elimination through a well-stirred liver model accounting for first-pass effect. The main predictors of nevirapine PK are the combined effect of SNP 516T>T and 983T>C, size (weight) and diurnal variation in hepatic clearance. Pharmacokinetic simulations are implemented on the final model to assess the effect of 516G>T|983T>C determined CYP2B6 metabolic subgroups and diurnal oscillation on nevirapine exposures and suggest adjustments to current treatment guidelines. The nevirapine pharmacokinetic/pharmacogenetic study is included as a full manuscript with the abstract, introduction, methods, results and discussion and its content has not been modified.

Chapter 7 (Publication/study 4) presents pharmacokinetic/pharmacodynamic study of nevirapine in African children treated with paediatric solid FDC tablets. The analysis is limited data from CHAPAS-3 and is performed using similar statistical methods to Chapter 5. In addition to describing nevirapine concentration/response relationship and identifying the predictors of virological response, it suggests exposure threshold most predictive of increased risk of non-suppression and liver elevations in African children. The nevirapine pharmacokinetic/pharmacodynamic study is included as a full manuscript with the abstract, introduction, methods, results and discussion and its content has not been modified.

Chapter 8 summarizes the results of the presented investigations in relation to the thesis' objectives and previously conducted studies. It discusses implications for treatment of paediatric HIV, presents limitations of presented research and identifies priorities for future of antiretroviral treatment in African children.

1.5 Coherence of thesis

I (Andrzej Bienczak) am the first author on all four papers included I this thesis. All four publications described above were prepared during my doctoral research conducted at the University of Cape Town under supervision of Doctor Paolo Denti and Professor Helen McIlleron, who jointly supervised my work while I was registered as a student at the University of Cape Town, including when I visited University of Liverpool as a non-degree seeking student. While at the University of Liverpool I conducted genotyping of children from CHAPAS-3 under supervision of Professor Andrew Owen (who is a co-author of two of four presented papers). The main data supporting this thesis, previously not analysed in terms of efavirenz and nevirapine, originates from CHAPAS-3. For the purpose of population pharmacokinetic modelling this data were enriched with data from pharmacokinetic sub-studies of CHAPAS1 and ARROW, which were conducted in similar populations. In addition to completing the genotyping, I conceptualised and conducted all analyses included in presented publications. Each publication addresses one or more of the thesis objectives and together with an introduction chapter (Chapter 1), a literature review (Chapter 2), justification of the choice of methodology (Chapter 3) and the concluding chapter (Chapter 8), form a coherent body of work which fully addresses the aims and objectives of my doctoral research. At the time of conceptualising the presented analyses, the combination of selected genetic polymorphisms had never been previously investigated in African children. The findings from the four presented publications provide data that may improve optimisation of dosing and outcome of treatment with efavirenz and nevirapine in this vulnerable population. This may ultimately contribute to “personalized medicine” for the HIV-infected children worldwide.

Andrzej Bienczak,

Cape Town, 6th of March, 2017

CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

The following chapter gives background information on human immunodeficiency virus (HIV) in terms of its epidemiology, disease pathogenesis and treatment. It outlines the current guidelines for treatment of HIV-infected children. It describes how the knowledge of pharmacokinetic (PK) and pharmacodynamics (PD) properties of a drug is paramount for treatment optimisation. It summarizes the efavirenz and nevirapine PK and PD, including pharmacogenetics, predictors of virological response and safety, with particular focus on the paediatric studies into PK and determinants of treatment response. It also gives summary of the published population pharmacokinetic models for both drugs.

2.1 Human immunodeficiency virus – epidemiology, disease and treatment

2.1.1 Epidemiology of HIV infection

Since the beginning of the epidemic in early 1980's, more than 78 million people have been infected with HIV and nearly half of them died from HIV related causes.[7] The most recent statistics from UNAIDS inform that by the end of 2015 there were 36.7 million people worldwide living with HIV, with over half of them living in eastern and southern Africa.[7] This region also accounts for 46% of new HIV infections globally.[7] The vast majority of HIV infected children live in sub-Saharan Africa (in 2014 91% of 3.2 million worldwide),[9] the statistics also consistently highlight this to be the area with the highest incidence of paediatric HIV infections.[2,7,9]

Figures from South Africa show that, at its peak in 2005, a maximum of 37-39% of child deaths could be attributed to acquired immunodeficiency syndrome (AIDS).[109] Children were first being diagnosed at an advanced stage of the illness with high viral loads, which contributed to a low treatment success rate.[12] A similar trend was also present in other African countries, with mortality rates particularly high within 90 days of treatment initiation.[14,47] On account of numerous national intervention programs in most of those countries aiming to scale up the prevention of mother-to-child transmission (pMTCT) of HIV and increase coverage of antiretroviral treatment (ART), the number of newly infected children is constantly decreasing.[2,8,109] In the last 5 years the incidence of new paediatric HIV infections worldwide declined by 50% (from 290'000 in 2010 to 150'000 in 2015),[7] and despite marginal numbers in developed countries, in 2015 in eastern and southern Africa 56'000 children were born with HIV and another 66'000 in western and central Africa.[7] Children with untreated infection rapidly progress to disease, especially in resource-limited settings where mortality is greater than 50% by 2 years of age.[110] Despite substantial increases in the HIV treatment coverage over the last few years,[8] in 2014 less than 25% of children needing ART in sub-Saharan Africa were receiving it.[9]

2.1.2 Pathogenesis of HIV infection

HIV is a letinovirus belonging to the family of retroviruses that causes a chronic infection in the host leading to progressive failure of the immune system called Acquired Immunodeficiency Syndrome (AIDS).[1] There are 2 types of HIV: HIV-1 and HIV-2, the first one being responsible for majority of infections word wide.[111] This thesis refers HIV to HIV-1 infection. The virus is transmitted through certain contaminated body fluids (such as blood, semen, pre-seminal, rectal and vaginal fluids and breast milk) when they come in contact with a mucous membrane or damaged tissue or are directly injected into the bloodstream. It can be spread in adults mainly through certain sexual activities or injection equipment and in children though mother-to-child transmission (during birth or through breast feeding). It infects vital cells of the human immune system such as CD4+ helper subset of lymphocytes T, monocytes, macrophages and dendritic cells.[1,112,113] As a consequence of the infection the number of CD4+ T-cells (crucial to regulating immune response)[114] visibly decreases compromising the function of immune system and its ability to detect pathogens leading its gradual failure.[112,115] Patients with late stages of AIDS are more susceptible to other opportunistic infections with other viruses, bacteria or fungi, as well as cancerous changes and their bodies eventually lose the ability to fight them.[1]

The lifecycle of HIV virus during the infection can be divided into a number of processes that are illustrated on Figure.2.1. After entering the host HIV virus fuses with CD4 lymphocytes through binding with CD4 and chemokine receptors (mainly CCR5).[116] Its content (including viral genome consisting of double stranded ribonucleic acid [RNA] and viral enzymes: reverse trascriptase [RT], integrase, ribonuclease and protease) is internalised in the affected cells.[117,118] In the next steps RT copies viral genome into deoxyribonucleic acid (DNA) creating proviral DNA, which is then integrated (by viral integrase) into the cell DNA, creating provirus.[116,119,120] At this stage, the infection is in a latent phase until the infected cells are activated. Cell activation stimulates the viral replication process through transcription and translation using cellular machinery. The proviral DNA is transcribed into messenger RNA, which is either spliced to generate RNAs to make viral proteins, or create new viral genome (full-length RNA), which are then transported to the cell plasma, where the viral proteases assemble them into virons.[116,119,120] Mature virons kill the T-cells through budding from the cell surfaces. This active replication process leads to progressive depletion of CD4 cells resulting in the aforementioned weakening of the immune system.[1]

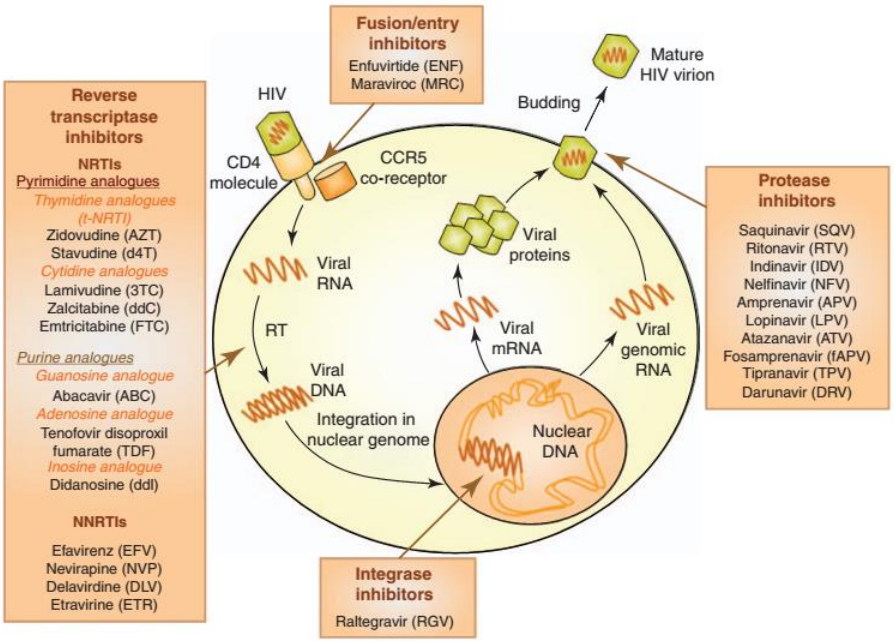

Figure 2.1 HIV lifecycle and the sites of action of antiretroviral drugs (from Apostolova et al.[121])[A]

Note: Explanation of the abbreviations can be found in the List of abbreviations and acronyms section preceding Chapter 1.

2.1.3 Pharmacological approach to HIV treatment

The primary aim of ART is to disrupt the viral replication process presented in Figure 2.1. The goal of treatment is to supress plasma viral load and restore and preserve immunologic function, which in consequence would lead to reduction in HIV-associated morbidity and mortality, prolonged survival, increased quality of life and reduce the risk of further HIV transmission.[122] It has been shown that durable suppression of viral replication can be achieved by combining a number of antiretroviral agents targeting different phases of HIV lifecycle.[123] Depending on their mode of action ARV drugs can be divided into 5 different classes:[123–125]

1. Entry inhibitors
   There are currently two drugs licensed in this group
   - Co-receptor CCR5 antagonist maraviroc (MRC) preventing the virus from attachment to co-receptor and preventing its entry into the host cell
   - Fusion inhibitor efuvirtide (ENF) which binds to viral glycoprotein gp41 preventing creation of an entry pore for the capsid of the virus and fusion with the host cell
2. Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs)
   NRTIs are the oldest and most widely used group of ARVs. Drugs from this group are competitive substrate analogues for reverse transcriptase. The active particle has a similar

[A] Reprinted from the *Trends Pharmacol. Sci.*, 32, 715–725, Apostolova, N. *et al.*, Mitochondrial interference by anti-HIV drugs: Mechanisms beyond Pol-γ inhibition. Page No. 716, Copyright (2011) with permission from Elsevier.

structure to nucleoside/tides present in the cell but they lack 3'-OH group in the ribose ring and if they are incorporated during DNA synthesis, they work as chain terminators. Examples include: stavudine (d4T), lamivudine (3TC), zidovudine (ZDV), abacavir (ABC), didanosine (ddI), and newer generation drugs such as emtricitabine (FTC) or tenofovir (currently available in two forms – tenofovir disoproxil fumarate [TDF] and alafenamide [TAF] with a more favourable safety profile).[126,127]

3. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
   NNRTIs are non-competitive reverse transcriptase inhibitors that cause steric hindrance by binding close to the active sites of the enzyme. They can be divided into 1st generation (with rigid structure with lower genetic barrier for developing resistance), e.g. nevirapine (NVP) and efavirenz (EFV); and 2nd generation (with more flexible structure and higher barrier for resistance), e.g. rilprivirine (RPV), etravirine (ETR).
4. Integrase inhibitors
   The newest group of ARVs inhibiting the viral integrase (catabolising the integration of viral DNA into cell DNA). This group can be characterised by the most favourable safety profile, fastest and most durable viral suppression. Currently available drugs from this category are: dolutegravir (DTG), elvitegravir (EVG) and raltegravir (RAL).
5. Protease inhibitors (PIs)
   PIs are competitive substrate-based inhibitors for viral protease. Despite high potency and high genetic barrier for resistance, PIs has been associated with a number of side effects, in particular metabolic abnormalities related to mitochondrial toxicity.[128,129] The most popular agents from this group are: lopinavir (LPV), atazanavir (ATV), amprenavir (APV), darunavir (DRV), fosamprenavir (fAPV) and tipranavir (TPV). Due to unfavourable PK properties majority of the listed drugs are given in combination with ritonavir (/r = PK boosting), which by inhibiting their metabolism enables reduction of dose of the other PI.

All current treatment guidelines advise that 1st line ART should consist of a combination of 2 NRTIs with a 3rd agent form a different class. According to most recent WHO recommendations preferred first line NRTI backbone in adults and adolescent should consist of a combination of TDF + FTC/3TC (alternative 1st line - ABC+3TC). The preferred companion drug is efavirenz and alternative regimens are based on DTG or nevirapine.[6] The choice of 1st line WHO regimens is guided not only by their efficacy and toxicity but also cost/benefit analyses and availability in all settings. In light of the recent results of ENCORE, showing similar efficacy of efavirenz 400mg once daily dose compared with the currently licensed 600mg[102,130] but improved treatment tolerability and hypothesised cost savings,[131] the WHO guidelines[6] were expanded to include both options.

Over the last few years a number of clinical trials in newer ARV agents stimulated changes in national formularies in resource-rich countries.[132,133] Due to demonstrated durable virologic efficacy, favourable tolerability and toxicity profiles, and ease of use the integrase inhibitor based regimens (in particular DTG) are the currently recommended first line option. Other first line or alternative recommendations include newer PIs (ATZ/r or DRV/r) and the new NNRTI RPV.[132,133] Efavirenz is still

listed as a viable treatment option. The preferred NRTI backbone consists of combination of FTC+TDF or TAF (ABC+3TC listed as alternative).

The recommendations in resource-limited countries are in line with the WHO guidelines[6] and list efavirenz 600mg/400mg+TDF+ETC (or efavirenz 600mg/400mg+ABC+3TC) as preferred first line, which is mostly dictated by availability of cheap, generic 3-in-1 formulations consisting of these drugs.

2.1.4 Treatment of paediatric HIV

Paediatric HIV treatment guidelines differ considerably from adult recommendations and differ further between age groups and geographical regions. Table 2.1 compares most recent paediatric recommendations from USA,[134] Europe,[135,136] South Africa[137] and WHO (2015)[6], and is complimented with WHO 2010 guidelines[11] (tested in CHAPAS-3).

The main reason for differences in HIV treatment guidelines between adults and children is that a number of new ARV drugs have never been tested in the paediatric population. For example, the only integrase inhibitor currently licensed for use in children is RAL[138,139] and although drugs from this group are the preferred ART component in resource-rich countries in adults,[132,133] their application in children in the same setting is limited. Another reason for differences between adult and paediatric formularies are the safety concerns for some of the newer drugs. For example TDF is licensed from 2 years of age but its actual use is limited through its negative effect on bone growth (especially in younger children)[140–142] and disruption of vitamin D metabolism.[143] TDF is currently advised for use only in adolescents and adults.[6,134,135] TAF is a promising alternative to TDF as it has shown to have a more favourable safety profile in adults and adolescent but to date it has not been tested in children.[126,144]

The choice of treatment in particular age groups is determined primarily by availability of age appropriate formulations and clinical evidence. The main change that occurred in the last few years was reclassification of nevirapine based regimens from preferred to alternative 1st line in all of the paediatric age bands. Results of study P1060 showed consistently that LPV/r based regimens were superior in terms of virological outcome, reduced risk of treatment discontinuation and death in children <3 years old.[62,145,146] Furthermore LPV/r protects better from development of NRTI resistance without compromising the efficacy of second-line PI-based regimens.[147,148] In the older paediatric age groups (>3 years) nevirapine was on the other hand replaced by efavirenz as a favoured 1st line ART option.[111] Even though the evidence of virological superiority of efavirenz over nevirapine is weak,[149–154] this choice aligns with the new recommended NRTI-backbone (ABC+3TC) allowing once daily dosage.[111] It has been speculated that once daily regimens might improve treatment compliance and

in consequence improve its outcome.[155–157] Furthermore co-treatment of tuberculosis infection does not require a regimen change or dose adjustment with efavirenz based ART.[74]

Despite being an alternative choice for 1st line ART, nevirapine is still widely used in sub-Saharan Africa for a number of reasons. Treatment with LPV/r might be challenging in resource-limited setting, as for the youngest children the main available formulation is a syrup which requires cold chain environment until the point of dispensing. Additionally it has poor palatability which can potentially compromise adherence and have unpredictable effect on drug's bioavailability. Nevirapine is a constituent of the first paediatric ART available as an FDC all-in-one tablet targeted to help overcome feasibility issues related to treatment with liquid formulations and multiple drugs and to lower treatment cost. This treatment option has a high acceptability - a study showed that young children and their caregivers preferred paediatric tablets to a syrup-based treatment.[158] Currently there is no similar all-in-one paediatric solid formulation containing efavirenz. Use of nevirapine was previously discouraged in children, who received this drug for prevention of mother to child transmission (pMTCT) but a number of studies showed similar treatment outcomes independent of prior exposure.[60,61] It has also been shown that children who achieved viral suppression on LPV/r based regimen can be safely switched to a nevirapine-based ART maintaining positive virological outcomes (maintenance of virological load below threshold of detection for the used assay).[58,59]

The local variations in the recommended 1st line ART in children are presented in Table 2.1 and show similar trends to adult recommendations – the choice of treatment in resource-limited setting is determined not only by the supporting clinical evidence but also the drug availability, the cost of treatment and its feasibility. WHO guidelines take into consideration all of those aspects and most local formularies in resource-limited setting are usually in line with those recommendations. The cost of ART is not a significant constraint in resource-rich setting where patients have access to newer, and more expensive alternatives. Those treatment options are not available in sub-Saharan Africa making optimisation of currently available paediatric regimens a priority.

Table 2.1 Comparison of current paediatric treatment guidelines

Age group (years)	WHO 2010[11]		WHO 2015[6]		PENTA 2015[135,136]		USA 2016[134]		South Africa 2015[137]
	Standard	Alternative	Preferred	Alternative	Preferred	Alternative	Preferred	Alternative	Standard
< 2	NVP[a]/ LPV/r + 3TC + ABC/d4T/ZDV	3TC + ABC + d4T/ ZDV	LPV/r + 3TC + ABC/ZDV	NVP + 3TC + ABC/ZDV	NVP/ LPV/r + 3TC + ABC[d]	NVP/LPV/r + 3TC + ZDV	LPV/r + 2 NRTIs[e]	NVP + 2 NRTIs[e]	LPV/r + 3TC + ABC
2 – 3	NVP + 3TC + ABC/d4T/ZDV						RAL/ LPV/r + 2 NRTIs[e]	NVP/RAL/ ATV/r + 2 NRTIs[e]	
3 – 6	NVP/EFV + 3TC + ABC/d4T/ZDV		EFV + 3TC + ABC	NVP/ EFV + 3TC/ FTC[b] + ABC/ZDV/TDF	EFV/ LPV/r + 3TC + ABC	NVP/DRV/r + 3TC/FTC[b] + ZDV/TDF	EFV/RAL/ATV/r /DRV/r /LPV/r + 2 NRTIs[e]	------	EFV[g] + 3TC + ABC
6 - 12					EFV/ ATV/r + 3TC + ABC	NVP/ DRV/r /LPV/r + 3TC/FTC[b] + ZDV/TDF			
> 12			EFV + 3TC/3TC + TDF	NVP/EFV/ EFV$_{400}$[c]/DTG + 3TC/FTC[b] + ABC/ZDV/TDF	EFV/ATV/r /DRV/r + 3TC/FTC[b] + ABC/TDF	NVP/LPV/r /RAL/DTG + 3TC + ABC	DTG/ATV/r / DRV/r/EVG/c + 2 NRTIs[e]	EFV/RPV/RAL + 2 NRTIs[e]	EFV[h] + 3TC + ABC

[a]Children with no prior or unknown exposure to NVP for pMTCT. [b]Only in combination with ABC or TDF. [c]Only in combination with TDF+FTC. [d]Plus ZDV if CNS involvement or high VL. [g]ABC+3TC+LPV/r based regiment for children who started it before 3 years. [e]Preferred NRTI backbone: <3 months - ZDV+3TC/FTC. >3months to <12 years – ABC/ZDV + 3TC/FTC. >12 years – TDF/TAF+3TC or ABC+3TC/FTC [h]If <40kg align with children, if >40 kg treat as adults (EFV+TDF+FTC)3TC – lamivudine, ABC – abacavir, ATV – atazanavir, d4T – stravudine, ddI – didanosine, DTG – dolutegravir, DRV – darunavir, EFV – efavirenz, EVG/c – corbicistat boosted elvitegravir, LPV – lopinavir, RAL – raltegravir, RPV – rilprivirine, TAF – tenovir alafenamide, TDF – tenofovir disproxil fumarate, ZDV – zidovudine

2.2 Pharmacological considerations and treatment optimisation

Pharmacokinetics (PK) is the study of how the body affects the drug and can be described through a number of processes that take place between when the drug enters and exits the system, those are: absorption, distribution, metabolism and excretion.[159] The drug can be described in terms of its PK parameters (primary and secondary), which characterise its properties, some of them are: clearance (CL), volume of distribution (V), absorption rate constant (ka), half-life ($t_{1/2}$), elimination rate constant (kel), maximum concentration and time when it is reached after administration of a dose (C_{max} and t_{max}), as well as it's trough concentration (C_{min} – concentration at the end of a dosing interval) and AUC (area under the curve – describing drug exposure within a dosing interval).[160]

Pharmacodynamics (PD) on the other hand is the study of how the drug affects the body and refers to the biochemical, physiologic, and molecular effects of drug interacting with the system. The PD effects of the drug can be classified as desirable or unwanted. The desirable effects are measured in terms of the drug's efficacy (in a clinical trial setting) or effectiveness (term more appropriate to real life setting) and are both related to its mechanism of action. The unwanted (or undesirable) effects are referred to a side effects or adverse effects (AEs) and are often related to off-target interactions of the drug with the body.

The main paradigm of pharmacology is that there is a relationships between drug dose, exposure and response, referred to as PK/PD relationship, which can be defined and quantified.[93] The response to a therapeutic agent can be affected by the variability in drug's PK but there can be other, independent factors affecting the treatment outcome. A drug's efficacy and safety determine its therapeutic index, which is a comparison between the amount of an agent that causes a therapeutic effect to the amount that causes toxicity. In practice a term which is more often referred to is the therapeutic target (or range), which is the range of drug concentrations (usually measured in the plasma) producing therapeutic response without causing any significant AEs in the patients. The lower cut-off for a therapeutic range is determined by lowest exposure providing acceptable treatment efficacy and the upper cut-off is defined by the drug's safety profile.

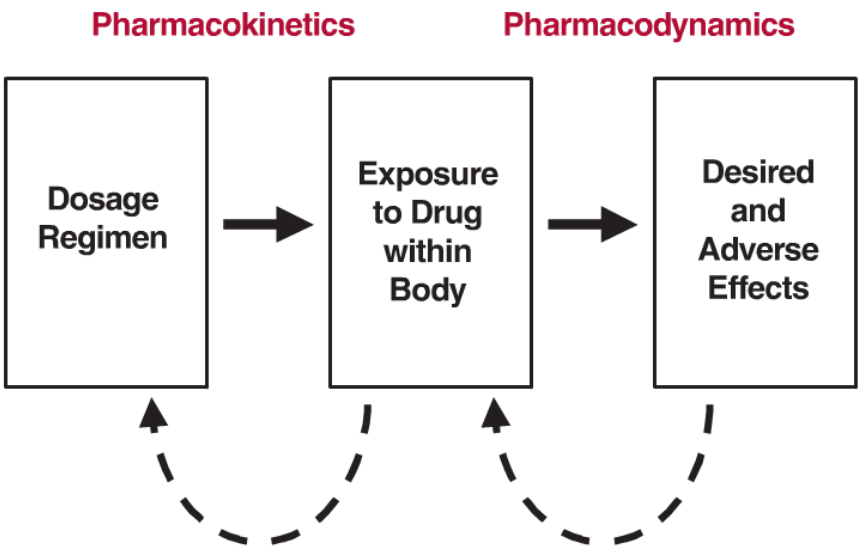

Figure 2.2 A rational approach to the design of a dosage regimen (from Rowland and Tozer[160])[B]

Note: The pharmacokinetics and pharmacodynamics of the drug are first defined. Then, response to the drug, coupled with pharmacokinetic information, are used as a feedback (dashed lines) to modify the dosage regimen to achieve optimal therapy.

It can be hypothesised that as long as the individual drug exposures remain within the therapeutic range the patient should have a successful treatment outcome. The objective of a rational approach to the design of dosing regimen (Figure 2.2) is to suggest a dosing strategy providing that individual drug concentrations remain within the therapeutic target during the dosing interval. Between subject variability is the source of different response to treatment between patients under the same dosing regimen. The aim of quantitative pharmacology, or pharmacometrics, is to identify sources of this variability affecting the drug response and to quantify them. Those sources could be certain demographic characteristics (i.e. weight, age or gender), inherent genetic traits (i.e. single nucleotide polymorphisms in genes coding metabolic enzymes affecting individual metabolic capacity), co-treatments (i.e. drug-drug interactions), environmental factors (i.e. malnutrition), or others. Based on that knowledge one could suggest treatment optimisation strategies adjusting the universal dosing regimen for known characteristics affecting individual drug response to ensure improvement in treatment outcome in the subpopulation containing those characteristics.

[B] Reprinted from the *Clinical Pharmacokinetics and Pharcodynamics, Concenpts and Applications*, Chapter 1: Therapeutic Relevance, Rowland, M. & Tozer, T. Page No. 4, Copyright (Lippincott, Williams & Wilkins, 2011) with permission from Wolters Kluwer Health.

2.3 Pharmacology of non-nucleoside reverse transcriptase inhibitors

There are four currently licensed NNRTIs but only two of them (efavirenz and nevirapine) are licensed for treatment in children < 12 years. Their pharmacological properties and studies that contributed to the current state of knowledge are discussed below.

2.3.1 Efavirenz

2.3.1.1 Pharmacokinetics

According to manufacturer's information after repeated administration of once daily 600mg dose in adults efavirenz has a steady state C_{max} = 4.07 ± 1.17 mg/L (29%) [mean ± S.D. (% C.V.)], which is reached 3 – 5 hours after administration, steady state C_{min} = 1.76 ± 1.01 mg/L (57%), and AUC = 58 ± 23 mg·h/L (40%).[63] Efavirenz has lower absorption at higher doses[161] (but linear PK within therapeutic doses[63]); the oral bioavailability is 40-45% without food,[63] and is 20% lower in liquid formations.[40,162] If taken with food absorption is increased up to 50%.[11] Efavirenz is highly protein bound (>99%) – mostly to albumin and although penetrates to central nervous system, the fraction that goes through blood-brain-barrier is low.[63] Population pharmacokinetic analyses (Tables 2.11 to 2.15 in Appendix to Chapter 2) have described efavirenz with a 2-compartmental distribution,[163–166] although in several studies based primarily on sparse data the 2nd compartment could not be identified.[66,167–172] High volume of distribution (combined V_{cent} and V_{per} ranging between 250 and 340L for average weight of about 70kg) indicates that despite high protein binding the drug penetrates into tissues (and confirms the CNS penetration).[170] Efavirenz ka is estimated to range between 0.14 and 0.6 [h^{-1}][66,71,103,167,168,170–173] and is described as a 1st order process (at dose of 600mg). Delays in drug absorption were previously modelled using a combination of a zero and 1st order process[170] or through transit compartments.[71,165]

Efavirenz is metabolised by the CYP system to inactive hyroxylated metabolites (Figure 2.3) and is a drug with relatively low extraction ratio.[63,174,175] The primary metabolite is 8-OH EFV (accounting for 77% of overall metabolism)[176] and corresponding principal catalyst is CYP2B6 with lessen contribution of CYP1A2, CYP3A5, CYP3A4 and CYP2A6.[63,175,177] The secondary metabolite is 7-OH EFV mainly produced though CYP2A6, this a minor metabolic pathway and becomes more important in patients with impaired function of CYP2B6.[174,177–180] Contribution of other 1st phase metabolites is negligible. In addition to primary oxidation 17% of 7-OH EFV can be further hydroxylated thought CYP2B6 to 7,8-dihyroxy EFV. In the 2nd phase efavirenz

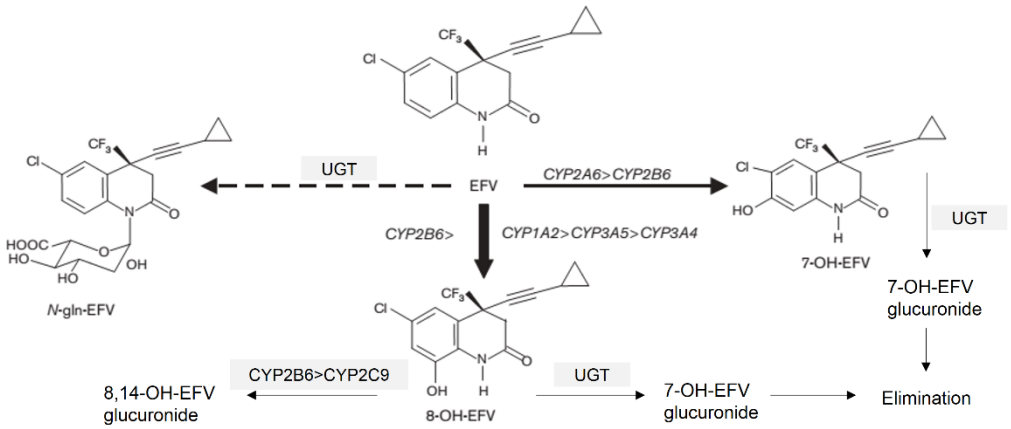

Figure 2.3 Suggested metabolic pathways for efavirenz with corresponding cytochrome P450 catabolic enzymes for main metabolites (based on di Julio et al.[174])[C]

undergoes glucuronidation (through uridine 5'-diphospho-glucuronosyltransferase - UGT), followed by its extretion.[63,180,181] By inducing CYP3A4, CYP2B6 and UGT1A1 pathways[63] efavirenz alters metabolism of other drugs as well as its own (it is an auto-inducer)[161,182] which leads to differences in clearance rates between single dose and at steady state (up to 150% higher at steady state).[164,166] The estimated steady state CL ranges between 8 and 11.7 L/h[168–170,172] and has also been described as a 1st order process, although three studies described it using a semi-mechanistic well-stirred liver model, allowing to account for 1st pass metabolism[71,172] or auto-induction.[166] Efavirenz clearance was shown to be increased by 19% during pregnancy.[71] The drug has a relatively long half-life (single dose $t_{1/2}$=52-76h, steady state $t_{1/2}$=40-55h),[63] which allows once daily administration. Efavirenz is excreted mainly in faces (both as metabolite and unchanged drug), approximately 14-34% is excreted really (primarily as metabolites).[161]

Numerous investigations showed that efavirenz PK exhibits high levels of between-subject variability reaching up to 120%[94] and coefficient of variation in drug's clearance up to 40-55%.[165,171] It has been attributed to a combination of various factors including biologic, exogenous and pharmacogenetic. Population pharmacokinetic investigations quantifying their effect on efavirenz PK are presented in Tables 2.11 to 2.15 in Appendix to Chapter 2. Efavirenz pharmacogenetic variability attributed to single nucleotide polymorphisms (SNPs) in genes coding enzymatic proteins for the drug's main metabolic pathways (Figure 2.3) have been identified as the main contributory factor.[171,173]

[C] Reprinted from the *Pharmacogenet. Genomics*, 19, 300–9, di Iulio, J. *et al*., In vivo analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. Page No. 301, Copyright (2009) with permission from Wolters Kluwer Health, Inc.

2.3.1.2 Pharmacogenetics

2.3.1.2.1 CYP2B6

The gene coding CYP2B6, the principal metabolic pathway for efavirenz, is highly polymorphic leading to up to 100-fold between-subject variability at enzymatic protein levels.[183] The frequencies of those polymorphisms vary significantly between populations (Table 2.2).[29,69,183–190] The most widely studied is the loss-of-function (LOF) polymorphism, 516G>T (rs3745274), which was first described by Lang *et al*.[191] and identified to be a point mutation at exon 4 leading to an amino-acid substitution in *CYP2B6* (Gln[172]His). Haas *et al*.[69,185] showed that this polymorphism was associated with differences in plasma exposures and frequency of CNS AEs but not virological outcome, and reported the 516TT variant allele to be more common in African-Americans (20%) than in European-Americans (3%).[69] A number of investigations confirmed those findings showing that this SNP is particularly prevalent in black Africans[180] with minor differences in recessive allele frequencies between ethnic groups (43% in black South Africans,[192] 42% in Tanzanians,[193] 31% in Ethiopians,[193] 48% in Zimbabweans[36] and 36% Ugandans[39]) and is associated with increased efavirenz levels observed in this population.[39,76,176,178,179,194,195] Population pharmacokinetic analyses estimated the 516GT genotype to cause a 23-50% reduction in efavirenz clearance and 516TT a drop of 54-75%.[36,169,173,185,196,197]

Table 2.2 CYP2B6 loss-of-function allele frequencies in different ethic groups (from Klein et al.[35])[D]

SNP	African-Americans	Ghanaians	Japanese	Taiwanese	Koreans	Caucasians
516G>T (rs3745274)	27.8%	48.8%	14.4%	14.1%	15.2%	25.5%
983T>C (rs28399499)	4.4%	6.6%	0%	0%	0%	0%
15582C>T (rs4803419)	0%	2.5%	0%	0%	0%	----

Another polymorphic *CYP2B6* variant allele affecting exposure to efavirenz is 983T>C (rs28399499). It was first associated with the differences in efavirenz metabolism by Klein *et al*.,[35] who sequenced it (g.18799 in exon 6) and reported the mechanism leading to impairment of the enzymatic protein (Ile[328]Thr). Klein *et al*.[35] hypothesised this SNP to be absent in Caucasian and Asian population (Table 2.2), which was later confirmed by Mehlotra *et al.*[188] The actual combined effect of SNPs 516G>T and 983T>C on efavirenz PK was first demonstrated by Wang *et al*.[29] and Wyen *et al*.[198] (who was also the

[D] Reprinted from the *Pharmacogenet. Genomics,* 15, 861–73, Klein, K. *et al*., Genetic variability of CYP2B6 in populations of African and Asian origin: allele frequencies, novel functional variants, and possible implications for anti-HIV therapy with efavirenz. Page No. 865, Copyright (2005) with permission from Wolters Kluwer Health, Inc.

first to report the effect of 983T>C recessive homozygosity). Ribaudo *et al*.[199] suggested that the combined effect of those SNPs on efavirenz PK can be expressed as a composite factor based on the combined number of minor allele polymorphisms (0, extensive metabolizer; 1, intermediate metabolizer; 2, slow metabolizer). Similarly to 516G>T the frequency of 983T>C recessive allele differs among different African ethnic groups, ranging between 5 – 17%.[71,76,189,193,195,199,200] It has been shown that despite having lower prevalence SNP 983T>C affects efavirenz PK to greater extent than 516G>T.[103,201]

CYP2B6 785A>G is another polymorphism reported in a number of investigations due to its linkage with both 516G>T[35,191] and 983T>C.[29] The effect of this SNP has been previously described on its own, as a separate allele (*CYP2B6*4*), showing to increase the CYP2B6 protein exporession,[29] or as a haplotype group in combination with 516G>T *(CYP2B6*6)* [35,191,202] *or* 983T>C *(CYP2B6*16*),[29] modifying their effect.[29,191] The described effect of 785A>G is low (in most studies not significant)[171] and for that reason most current investigations quantify the effects of 516G>T or 983T>C separate and not as *CYP2B6*6* and *CYP2B6*16* haplotype groups.[180]

In a recent genome wide association study Holzinger *et al*.[176] identified that the effect of the two aforementioned polymorphisms (516G>T and 983T>C) is further modified by SNP 15582C>T (rs4803419, Thr[168]Ile) giving 11 possible combinations of *CYP2B6* allele variants in the three genetic locations (homozygosity for any one of those three polymorphisms precludes the presence of another two). Based on those combinations authors suggested that individuals can be classified into different metabolic subgroups for efavirenz (Table 2.3).

The findings by Holzinger *et al.*[176] were recently replicated in a cohort of South African adults and children[76] but the effect of SNP 15582C>T on efavirenz PK wasn't confirmed in others.[71,189,195,203] Admittedly SNP 15582C>T does not improve the ability to predict very high efavirenz concentrations (as 15582T cannot be present among individuals who already have two variant alleles at *CYP2B6* 516G>T and/or 983T>C) but only in groups having low exposures,[176] and despite proving significant in the South African study the investigators concluded this effect was negligible in comparison to 516G>T and 983T>C.[76]

Alternative classification of metaboliser subgroups based on combination of SNPs 516G>T and 983T>C alone was recently proposed by Dooley *et al*.[71] in a study in South African women (Table 2.3). Due to significant differences in clearance rates (up to 10-fold differences between the two most extreme subgroups) the investigators suggested that individuals having homozygous variant at position 983T>C should consist a separate group of ultra-slow metabolisers (USM).

Table 2.3 Metabolic subgroups for efavirenz based on CYP2B6 allel variants proposed in different investigations

MET	Holzinger et al.[176]	Dooley et al.[71]			Dickinson et al.[103]**		
	15582C>T| 516G>T |983T>C	516G>T| 983T>C	CL* [L/h]	C_{min} [mg/L]	516G>T| 983T>C| CYP2A6*9B|*17	CL [L/h]	C_{min} [mg/L]
EM	Homozygous wildtype at 516G>T and 983T>C and wild type or heterozygote for 15582C>T (CC|GG|TT or CT|GG|TT)	Homozygous wildtype for both SNPs (516GG|983TT)	18.6	1.33	Homozygous wildtype for CYP2B6 SNPs with combinations of CYP2A6 alleles (GG/TT/CC/CC, GG/TT/CC|CT or TT, GG|TT|CA or AA|CC, GG|TT|CA or AA|CT or TT)	12.4	1.28
IM	Homozygous wildtype at 516G>T and 983T>C with homozygous variant at 15582C>T or one variant allele at 516G>T or 983T>C (TT|GG|TT or CC|GT|TT or CC|GG|CT or CT|GT|TT)	One variant allele at either of the positions (516GT|983TT or 516GG|983CT)	11.0	2.03	Homozygous wildtype 516G>T and combinations of CYP2A6 alleles with heterozygous or homozygous variant 983T>C or heterozygous variant 516G>T with 983T>C homozygous wildtype and combinations of CYP2A6 alleles (GG|TC or CC|CC|CC, GT|TT|CC| CC, GT|TT|CC|CT or TT, GT|TT|CA or AA|CC, GT|TT|CA or AA|CT)	8.93	1.94
SM	One variant allele at either 516G>T or 983T>C or homozygous variant at 516G>T (CC|TT|TT or CC|GT|CT or CC|GG|CC)	One variant allele at either of the positions or homozygous variant at 516G>T (516GT|983CT or 516TT|983TT)	4.79	6.11	Heterozygous variant 516G>T with heterozygous or homozygous variant 983T>C, or homozygous variant 516G>T with homozygous wildtype 983T>C with combinations of alleles (GG|TC or CC| CC|CT or TT, GG|TC or CC|CA or AA|CC, GG|TC or CC| CA or AA|CT, GT|TC or CC|CC|CC, GT|TC or CC|CC|CT or TT, GT|TC or CC|CA or AA|CC, TT|TT|CC|CC, TT|TT| CC|CT or TT, TT|TT|CA or AA|CC, TT|TT|CA or AA|CT)	3.55	6.24
USM	NA	Homozygous variant at 983T>C (516GG|983CC)	1.56	17.42	NA	NA	NA

*expressed as apparent hepatic clearance, **values corresponding to efavirenz daily dose of 600mg

In addition to the listed *CYP2B6* polymorphisms two novel SNPs have been recently linked with efavirenz concentrations in Thai patients - 18492T>C and 21563C>T.[204–206] Sukasem *et al.*[206] suggested that the composite effect of SNPs 516G>T, 785A>G and 21563C>T could be defined as a new *CYP2B6*-GAC haplotype and reported frequencies of 36.5%, 40.4% and 23.1% for non-GAC, GAC heterozygous and GAC homozygous genotypes, respectively, with corresponding average efavirenz concentrations of 1.94 mg/L, 2.69 mg/L and 5.54 mg/L. In order to exclude possible confounding effect of other SNPs the new associations were evaluated independently in patients who carried CYP2B6*1/*1 (516GG|983TT) in two separate studies.[204,205] They reported consistently that 18492T>C (but not 21563C >T) was significantly associated with lower efavirenz concentrations and that individuals carrying variant allele were at increased risk of virological failure.[204,205] Lastly, SNP *CYP2B6* 136A>G (rs35303484) was associated with lower efavirenz clearance (20% reduction) for in Ugandan individuals by Mukonzo *et al*.,[39] but this association was not replicated in other studies.

2.3.1.2.2 Accessory pathways

Efavirenz accessory metabolic pathways have also been shown to be highly polymorphic[180] but due to lower contribution to the overall biotransformation they are less studied. A number of investigations showed that those polymorphisms become more important in slow CYP2B6 metabolisers where the clearance is diverted towards the 7-OH EFV pathway catalysed primarily by CYP2A6.[171,174,177–179] Di Iulio *et al*.[174] showed in an *in vivo* analysis that dual slow CYP2B6 and CYP2A6 metabolism can lead to extremely high efavirenz concentrations, which was replicated by Arab-Alameddine *et al*.[171] Haas *et al*.[185] reported that *CYP3A5* 6986AA homozygotes had a 10% increased efavirenz clearance. In a study in Ghanaian patients *CYP2A6*9B* (-48T>G) and/or *CYP2A6*17* carries had on average 1.8 higher efavirenz concentrations comparing to non-carriers[178] and the drug exposures were further altered by a polymorphism UGT 735A>G (*UGT2B7*1a*).[179] *CYP2A6 -48T>G* was predictive of efavirenz concentrations in another Ghanaian cohort but only in a univariate analysis and no longer after accounting for *CYP2B6* 516G>T and 983T>C, suggesting that those association might not be independent of CYP2B6 polymorphisms.[207] None of those findings were confirmed in a genome wide association study by Holzinger *et al*.[176] and to verify them Haas *et al*.[207] conducted an investigation in a group subjects with slow 516G>T|983T>C genotype. The authors found that CYP2A6 -48T>G and UGT2B7 735A>G recessive homozygosities were predictive of further elevations in efavirenz exposers. Recently Dickinson *et al.*[103] suggested efavirenz metabolic subgroup classification based on combination of SNPs 516G>T and 983T>C in *CYP2B6* and *CYP2A6*9B/*17* (Table 2.3).

2.3.1.2.3 Nuclear receptors

CYP450 enzymes activity is regulated by a number of nuclear receptors (Figure 2.4). Nuclear receptors are ligand-activated transcription factors that control various cell and organism functions on a molecular level by up- or down-regulating expression of proteins.[78] CYP450 activity is influenced mostly by pregnane (steroid) X receptor (PXR) and the constitutive androstane receptor (CAR).[78–80] As a consequence, additionally to SNPs in genes coding CYP450 enzymes, also polymorphisms in genes coding the aforementioned receptors (*NR1*|*2* for PXR and *NR1*|*3* for CAR) are hypothesised to indirectly impact efavirenz PK.[81,82] SNPs in nuclear receptors are also shown to be prevalent in different frequencies in various ethnic groups.[83,84] The number of investigations reporting significant effect of the polymorphisms in nuclear receptors on efavirenz PK is scarce, and so far they were only confirmed for *NR1*|*3* (CAR) in one study in Chilean patients (rs2307424 C>T)[208] and in several studies in South African individuals (rs3003596T>C).[84,189,195] Swart *et al*.[84] showed that the effect of polymorphisms in *NR1*|*3* might be more prominent in slow metabolisers for CYP2B6. Those associations were not replicated in the majority of other investigations.[76,176,207,209]

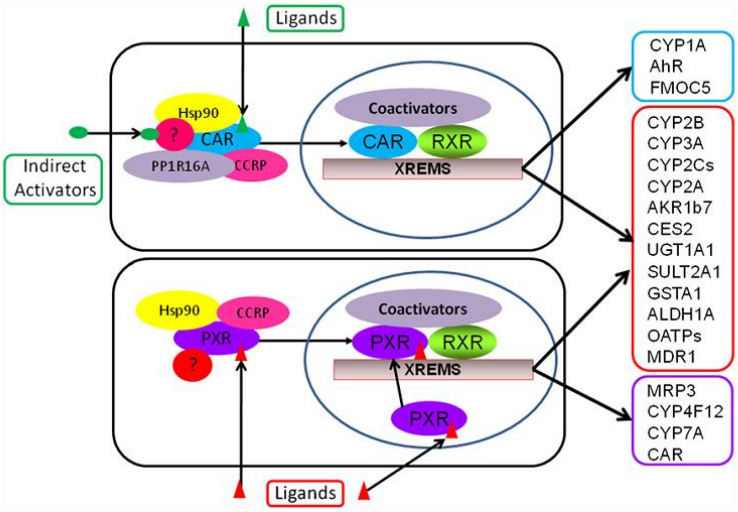

Figure 2.4 Schematic illustration of the activation mechanisms and target genes of CAR and PXR (from Tolson et al.[210])[E]

Note: CAR can be activated by either direct (ligand binding) or indirect mechanisms, while activation of PXR is purely ligand dependent. CAR and PXR shared target genes are grouped in a red box, CAR-specific targets in a blue box, and PXR-specific targets in a purple box.

[E] Reprinted from the *Adv. Drug Deliv. Rev*., 62, 1238–49, Tolson, A. H. *et al*., Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. Page No. 1240, Copyright (2010) with permission from Elsevier.

2.3.1.2.4 Transporters

Variability in efavirenz PK has been previously linked to polymorphisms in *ABCB1* (ATP-binding cassette transporter gene or multidrug-resistance transporter gene) coding P-glycoprotein (P-gp). P-gp itself has an important role in transportation of a number of different substrates (including some ARVs) at a compartmental and cellular level (Figure 2.5). It is abundant in the intestines (regulating drug entry into the body), in cell membranes (regulating drug entry into the cells), and in the apical membrane of many other epithelial barriers, e.g. blood-brain barrier (regulating the inter-compartment drug distribution).[211] Decreased expression of P-gp (Figure 2.5b) leads to increased drug concentrations in plasma (due to reduced intestinal reverse efflux - E) causing increased intracellular concentrations (on Figure 2.5b in CD4+ Lymphocytes). This effect was first reported by Fellay *et al*.[212] who associated higher efavirenz concentrations leading to significantly larger CD4 increases in patients with *ABCB1* 3435TT genotype. It an be speculated that the disparities in some findings could be a consequence of differences in accuracy of intracellular drug concentration measurements and reproducibility of assays. The association between *ABCB1* 3435C>T and exposure to efavirenz was replicated in a number studies,[39,168] while others related the *ABCB1* 3435C>T recessive homozygosity only to better treatment response (decreased likelihood of virologic failure[185] or higher increase in CD4-cell count[213]). Population pharmacokinetic investigations by Csajka *et al*.[168] and Mukonzo *et al*.[39] reported that the observed differences in efavirenz plasma exposures for *ABCB1* 3435TT could be attributed to differences in oral bioavailability (20% increase). What makes this finding controversial is that it wasn't confirmed in others studies,[189,193–195,199,209,214,215] and in fact a number of *in vitro* investigations found no association between P-gp expression and efavirenz intracellular concentrations.[216–218] It was hypothesised though that the effect of *ABCB1* polymorphisms might be more pronounced on the intra-cellular drug concentrations than ones observed in plasma or cerebrospinal fluid (CSF).[219]

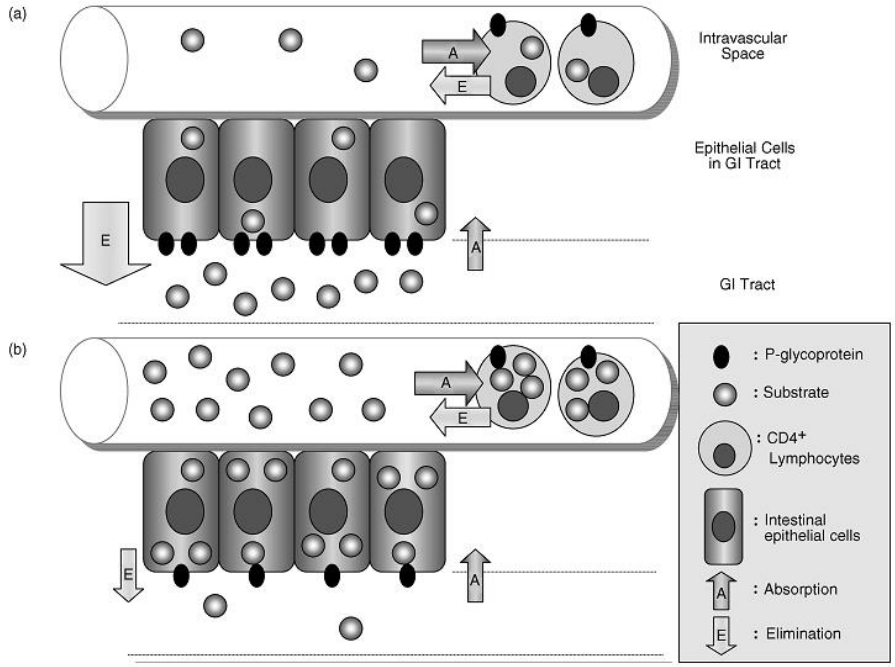

Figure 2.5 Hypothesis for differential expression of P-glycoprotein (P-gp) and drug concentrations of P-gp substrate in plasma (from Saitoh et al.[215])[F]

Note: (a) Normal expression of P-gp (b) Reduced expression of P-gp

A recent investigation by Swart *et al*.[220] in South African patients associated differences in efavirenz concentrations with two new SNPs in *ABCB1*: 1236C>T and 4036A>G (only 1236C>T remained significant after correction for *CYP2B6* 516G>T|983T>C and *N1|3* 8784T > C).[195] The finding for *ABCB1 1236C>T* hasn't been replicated since but allele *ABCB1* 4036G has been associated with increased efavirenz levels in studies in South Africans,[189] Ethiopians,[193] Tanzanians[193] and in Ugandans[194] (in all studies 4036A>G was an significant predictor after correction for *CYP2B6* 516G>T|983T>C). Mukonzo *et al*.[194] estimated that *ABCB1* 4046A>G variant allele carriers has a 22% increased F1. The mechanism for the effect of *ABCB1* polymorphisms on the PK of NNRTIs remains uncertain.

2.3.1.3 Other predictors of variability in the efavirenz pharmacokinetics

Other covariates identified to effect efavirenz PK are: race, sex, weight, different formations and other drugs. Several investigations reported consistently higher efavirenz exposures among black, particularly African individuals,[66,165,168,172,221,222] none of those studies though looked into the pharmacogenetic predictors and it is currently known that the reported differences were due to different prevalence of SNPs between populations.[69,171]

[F] Reprinted from the *AIDS*, 19, 371–380, Saitoh, A. *et al*., An MDR1-3435 variant is associated with higher plasma nelfinavir levels and more rapid virologic response in HIV-1 infected children. Page No. 377, Copyright (2005) with permission from Wolters Kluwer Health, Inc.

A population pharmacokinetic meta-analysis by Barrett *et al*.[164] reported 10% lower clearance in women relative to men, nonetheless this finding was questioned due to disproportion in distribution of sexes in the analysis (the population consisted of only 13% females). In a study by Burger *et al*.[221] women had 30% higher efavirenz concentrations, however the investigators did not account for the effect of *CYP2B6* polymorphisms and the majority of women under investigation were of African origin. The effect of sex was also detected in analysis by Nyakutira *et al*.[36] (30% reduction in CL for women) and Mukonzo *et al*.[39] (twice increased volume of distribution attributed to higher body fat in females) but it hasn't been replicated in other studies making it inconclusive. Weight has been also shown to affect efavirenz PK[71,167,222,223] but currently it is widely known that size affects the clearance and the volume of distribution of most drugs and is accounted for through allometric scaling.[224,225]

Efavirenz PK was speculated to be decreased by a concomitant use of rifampicin based TB treatment leading to recommendations of dose increase in patients weighing >50kg from 600 to 800mg.[226,227] This was not confirmed in other studies, however some of them showed high variability and unpredictability of observed efavirenz concentrations during TB treatment,[228,229] others conversely that rifampicin decreased efavirenz clearance leading to increases in average drug exposures.[222] More recent investigations show that the direction of this effect depends on individual *CYP2B6* genotype.[52,230] Isoniazid, another component of 1st line TB treatment, was linked with elevated efavirenz concentrations through inhibitory effect on its accessory metabolic pathways.[74,170,203] This effect is concentration dependant and is particularly high for individuals being slow *CYP2B6* metabolisers and slow *NAT2* acetylators (who have particularly high isoniazid levels).[170] The current consensus is that concomitant TB treatment does not require efavirenz dose adjustment.[52,170,203,231]

Lastly, zidovudine, a common antiretroviral companion drug, was associated with 25% reduction in efavirenz concentrations.[222] While an *in vivo* investigation showed that both drugs share a metabolic pathway (UGT)[181] giving a possible mechanism for this interaction, it was not confirmed in other studies. Furthermore, UGT is an accessory metabolic pathway for efavirenz and the relevance of finding is questionable and limited.

2.3.1.4 PK/PD relationship and therapeutic targets

Literature shows inconclusive information about the concentration – response relationship for efavirenz. The PK/PD relationship was first described by Joshi *et al*.[232] who reported that the risk of virological failure decreased with increasing efavirenz concentrations and that individuals with trough concentrations above 1.1 mg/L were at lower risk of treatment failure comparing to patients with exposures below that threshold (Table 2.4). Marzolini *et al*.[94] applied logistic regression to mid-dose

efavirenz concentration data obtained from 130 patients followed up for 3 – 18 months (up to 8 repeated visits per patient every 3 months) identifying that a similar efficacy cut-off (1 mg/L). This finding was later confirmed in a larger group of patients by Csajka *et al*.[168] The data from the two latter studies was recently pooled together and re-analysed by Siccardi *et al*.[233] and derived logistic regression model was applied to predict treatment outcome under different dosage scenarios.

A number of additional studies aimed to characterise PK/PD relationship for efavirenz, some of them suggesting alternative efficacy targets (Table 2.4), but a number of them failed to detect a significant association between drug exposures and treatment outcome.[103,105,209,234] Van Leth *et al*.[234] showed that in treatment adherent patients (on treatment for at least 95% of their follow-up) the concentration-effect relationship was very weak and the sensitivity of efficacy cut-offs was very low, suggesting that adherence had a more significant effect on treatment outcome than the actual plasma exposure levels. In a study in Ugandan patients Mukonzo *et al*.[194] failed to detect a significant association between efavirenz mid-dose concentrations and treatment outcome in terms of CD4 and/or HIV RNA levels but the investigation had a relatively small number of patients. The paediatric investigations contribution to the field are discussed in Chapter 2.3.1.7.5.

A lot of controversy surrounds the current efavirenz efficacy threshold of 1mg/L.[94,95] Firstly, the 1 mg/L threshold is considerably higher than the *in-vitro* protein binding–corrected efavirenz 95% inhibitory concentration (IC95) of 0.05 mg/L.[64] Secondly, recently resurfaced results of the efavirenz phase 2 dose finding study showed that all tested treatment options (200 mg, 400 mg and 600 mg) had a comparable efficacy.[235] The maximum effective dose of 600 mg was chosen to avoid the risk of emergence of resistance mutations but the results suggest that exposures much lower to current therapeutic target could provide adequate virological suppression.[130] This hypothesis was recently tested in study ENCORE1,[102,103] which proved that the standard 600mg efavirenz dose in adults can be reduced to 400mg daily without loss of efficacy. The extensive PK/PD analysis of the analysis of ENCORE1 data by Dickinson *et al*.[103,167] revealed that even though the correlation between exposure to efavirenz and virological suppression was present at 48 weeks of treatment, the confidence intervals for the effect were very wide, and the association were no longer present at week 96. The authors challenged the validity of the 1 mg/L cut-off suggested by Marzolini *et al.*[94] in prediction of virilogical outcome,[94] which in fact in a recent South African study was reported to be lower (Table 2.4).[236] Lastly the targed suggested by Marzolini *et al*. was derived in a very heterogeneous population comprising very heterogeneous population (including patients with prior exposure to other ARTs and ones who failed on previous treatment regimens) and some combination therapeutics, which are currently no longer used in HIV treatment and were replaced by more potenet companion drugs.

Table 2.4 Previously published efavirenz therapeutic targets

Pop	Ref (Year)	Target	Method	VL [copies/mL]	n
Adults	Joshi *et al*.[232] (1999)	C_{min} > 1.1 mg/L	No information	No information	----
	Marzolini *et al*.[94] (2000)	C_{TDM} = 1 – 4mg/L[a]	Logistic regression	400	130
	Csajka *et al*.[168] (2003)	C_{TDM} = 1 – 4mg/L[a]	logistic regression	400	235
	Gonzalez de Requena *et al*.[237] (2004)	C_{min} >3mg/L[b]	No information	50	48
	Stahle *et al*.[238] (2004)	C_{TDM} = 2.2 – 4.1mg/L	Cumulative distribution of concentrations	50	68
	Van Leth *et al*.[234] (2006)	C_{min} > 1.1 mg/L AUC > 40 mg*h/L	Cox model	50	800
	Orrell et al.[236] (2016)	C_{TDM} > 0.7 mg/L	Cox model + likelihood profiling	400 (at week 16)/ 40 (week 48)	180
Children	Starr *et al*. [96,239] (1999, 2002)	AUC = 60 – 120 mg*h/mL[c]	Exposure target extrapolated from adults	50, 400	57
	Brundage *et al*.[101] (2003)	AUC > 59 mg*h/mL[d]	Pre-defined + Cox model	400	50
	Hirt *et al*.[32] (2003)	C_{min} > 1.1 mg/L AUC > 51 mg*h/L	Pre-defined + Fisher's exact test	300	48
	Fletcher *et al*.[100] (2007)	AUC > 49 mg*h/mL[d]	Pre-defined + Chi-square	400	50
	Bouzza *et al*.[240] (2013)	C_{min} > 3.3 mg/L	HIV dynamic model	300	49

[a]Mid-dose concentration (8-20h post-dose, TDM). [b]Adults who failed on NVP. [c] Cut-off chosen to match adult exposures observed in Phase II study. [d]Most predictive lower cut-off.

Similar to other ARVs, efavirenz treatment efficacy has been shown to be affected by treatment adherence (in particular so called drug holidays - ≥48h periods of unplanned drug cessation), depression, younger age and baseline mutations,[241,242] but none of those predictors was as strongly associated with increased risk of treatment failure as suboptimal drug concentrations.[94,168,232,234,238]

2.3.1.5 Safety

As outlined in Chapter 2.3.1.4 the therapeutic target for a drug is defined by the lowest exposure providing acceptable treatment efficacy and upper cut-off is defined by drug's safety profile. For

efavirenz the sub-therapeutic concentration have been associated with increased risk of virological failure (discussed in previous chapter) and higher exposures with central nervous system (CNS) toxicities.[69,94,168]

Early studies in efavirenz showed that 20-40% of patients developed CNS toxicities with variable severity and duration, which led to treatment discontinuation in some of the individuals.[243,244] CNS disturbances ranged from dizziness to hallucinations, including headaches, problems with concentrations, depression, nightmares and insomnia.[63,245] Marzolini *et al*.[94] showed that severity of those side effects was concentration dependant and that they were 3 times more frequent in patients with efavirenz concentrations > 4mg/L. The concentration-dependant association with CNS AEs has been replicated in a number of investigations, which showed an increased incidence in slow CYP2B6 metabolisers[69,186,192,199] and black Africans,[246–248] with particularly high dug concentrations. It has been shown that the incidence of neuropsychiatric adverse events decreases with time, which has been associated with building of tolerance.[69] This could also explain less clear association with efavirenz concentrations and CNS AEs in some of the studies.[168] The severity of reported symptoms differs between studies between mild CNS manifestations such as insomnia, fatigue[69,186,192] to severe such as confusion, seizures and severe psychosis.[248,249]

The differences in prevalence of neuropsychiatric AEs between reports could result from underreporting in studies not utilising neuropsychological examinations. A recent study in predominantly Italian patients showed a high prevalence of HIV-associated neurocognitive disorders in apparently asymptomatic HIV+ individuals treated with efavirenz.[250] This has been confirmed in a systematic review speculating that efavirenz intake is associated with worsening of cognitive functions.[251] Despite the constrains related to detecting and reporting efavirenz associated neurological side effects studies consistently report average drug concentrations in individuals not experiencing similar AEs to range between 2.4 and 3.7 mg/L.[245] Conversely neurological side effects (resulting from high efavirenz exposures) were the cause of treatment discontinuation in 54% of patients in a Scandinavian cohort (half of them changing treatment after 12 months indicating persistence of symptoms).[252] In 90% of them after efavirenz discontinuation the unwanted symptoms subsided.[252]

High efavirenz concentrations related to slow *CYP2B6* metaboliser genotype have also been associated with high bilirubin levels[253] and increased risk of liver injury in case reports[254] and cohort studies.[255,256] The mechanisms underlying the efavirenz-related hepatotoxicity is currently not known.

2.3.1.6 Optimisation of efavirenz dosage

The aim of efavirenz dosage optimisation is to ensure individual patient exposures fall within the therapeutic range of 1-4mg/L in order to reduce the risk of sub-therapeutic concentrations associated with virological failure and prevent supra-therapeutic concentrations putting patients at risk of developing CNS AEs. The first treatment personalisation strategy was suggested by Csajka *et al*.[168] All variability in Csajka's pharmacokinetic model was allocated to bioavailability following an assumption that no matter what its true source is, it would lead to change in the individual value of bioavailability (F_{ind}). The average predicted efavirenz trough concentration (at steady state after daily administration of 600mg and population average bioavailability value assumed to be F_{pop}=1) was 1.87 mg/L. Calculating the ratio of model estimated F_{ind} and F_{pop} would allow adjustment of dose to provide more optimal exposure, i.e. for an individual with F_{ind}=1.4 the dose should be reduced by 1/1.4 (he should receive 0.7x standard dose). Such dose optimisation strategy has several limitations. It assumes the variability is constant over time, whereas a number of publications show that it's highly occasion dependant, which could lead to wrong conclusions if optimisation would be based on a single efavirenz sample. Furthermore, it does not allow a priori optimisation of dose before the start of treatment and it has been shown that the CNS AEs are particularly severe in the first weeks after treatment start.

An approach that would allow a priori prediction of individual efavirenz concentrations and dose adjustment before treatment start is based on individual patient *CYP2B6* genotype. First such approach was suggested by Nayakutira *et al*.[36] advising that efavirenz dose for poor metabolisers (516GG) could be reduced to 400mg and an even greater dose reduction (down to 200mg) was proposed by Arab-Alameddine *et al*.[171] Cabrera *et al*.[169] suggested the efavirenz dose should be reduced by 33% for each dysfunctional 516GT allele (516GT – 400mg, 516TT – 200mg), a dosing recommendation also suggested by Sanchez *at al*.[173] and Siccardi *et al*.[233] Recently a new dosing algorithm was derived in Chinese patients suggesting most optimal daily doses of 550 mg, 350 mg, and 100 mg for the 516GG, 516GT, and 5146TT populations, respectively.[257] This dosing algorithm was based on simulations in population pharmacokinetic studies and data on its implementation in a clinical setting and effect on treatment effectiveness and safety is limited. Of note is that none of the previously genotype based dose optimisation strategies accounted for the effect of SNP 983TC.

In a Japanese study Gatanaga *et al*.[258] successfully implemented dose reduction from 600 mg to 400 mg daily in 11 patients slow CYP2B6 metabolisers (516GG) with persistently high efavirenz concentrations (>6 mg/L), and in 7 of them the dose was subsequently reduced to 200 mg while maintaining virological suppression < 50 copies/ml. There are also case reports of similar successful dose reductions in patients with *CYP2B6* 516GG genotype.[259,260] A successful dose reduction guided by

efavirenz TDM concentrations was implemented in a small Swiss cohort of 13 patients.[261] Patients with efavirenz mid-dose concentrations between 75th and 95th percentile had the dose reduced to 400 mg and one with concentrations >95th percentile 200mg. All patients maintained virologic suppression <50 copies/ml and subsequent genotyping showed that all but one had CYP2B6 516TT genotype (one had CYP2B6 516GT). Similar TDM guided dose reduction strategy was also implemented in a small cohort of Italian patients.[262] In addition a recent modelling and simulation analysis showed average lifetime efavirenz treatment cost with a current standard dose of 600mg is 18500 USD higher than a genotype guided dosage, leading to incremental cost–effectiveness ratio >100,000 USD/QALY when comparing both treatment options.[263]

Re-analysis of the initial phase 2 efavirenz dose-ranging study showed no difference in efficacy between 400mg and 600mg doses.[264] Reduction of adult efavirenz dose from 600mg to 400mg could lower the cost of treatment by 30% and was estimated to bring savings of US$16 per person in a resource-limited setting, leading to an overall saving of US$192 million per year,[265] which could contribute towards increasing treatment coverage in low-income countries. This led to design and roll out of ENCORE1 study, which showed that such efavirenz dose reduction in adults, when combined with tenofovir and emtricitabine, could provide reduced incidence of side effects without compromising treatment efficacy. Such universal dose reduction has been widely discussed and it was highlighted that it might be not appropriate in all populations considering limited information in pregnant women and during TB co-treatment.[266] It was also questioned that two thirds of ENCORE1 participants were of African or Asian origin, where the prevalence of LOF CYP2B6 polymorphisms is particularly high.[267] Recently, pharmacokinetic simulations showed that reduction of efavirenz dose to 400mg in individuals 516GG might lead to underdosing in a larger proportion of patients than under current dose of 600mg and a dose of 500mg was suggested as most optimal in this subgroup.[267]

2.3.1.7 Paediatric investigations

The number of paediatric investigations describing efavirenz PK and sources of its variability is unsurprisingly limited what results from constraints of conducting clinical research in children outlined in Chapter 3.2.1. The majority of these studies include small numbers of patients and are based on predominantly sparse data. The number of PK/PD investigations (exploring association between efavirenz concentrations and treatment outcome) is even scarcer. The paediatric literature on efavirenz is further complimented by (predominantly comparative) studies evaluating treatment effect in terms of efficacy and safety (Tables 2.6 and 2.7). The overview of the paediatric studies in efavirenz is presented below (paediatric population pharmacokinetic analyses are additionally summarised in Tables 2.11 and 2.12 in Appendix to Chapter 2).

The product label for Sustiva (efavirenz originator drug) was recently extended in the USA[268] and Europe[269] to children as young as 3 months (weighing at least 3.5kg, Table 2.5), nonetheless WHO guidelines still recommend efavirenz should not be used in children < 3 years of age (Table 2.8).[6]

2.3.1.7.1 Early studies

The data leading to paediatric approval of efavirenz was provided by Pediatric AIDS Clinical Trials Group (PACTG) 382 study published by Starr *et al*. in 1999[96] (for solid formulations) and 2002[239] (for liquid formulation). Both studies proved high efficacy of efavirenz in combination with nelfinavir and one or two NRTI's (61% of children reached HIV RNA levels of <400 copies/ml, and 53% had <50 copies/ml in an intent-to-treat analysis in the first study and in the second 58% and 53%, respectively). Studies showed that efavirenz based treatment in children was well tolerated and that the CNS AEs previously observed in adults were less severe and less frequent. The first study in solid formulations reported high prevalence of skin rashes (occurring predominantly within 2 weeks of start of treatment), a finding which was not replicated in other trials. In those paediatric dose finding studies the initial efavirenz dose was derived to match adult exposures (Table 2.4) and calculated through allometric scaling ($600\ mg \bullet \left(\frac{\text{weight [kg]}}{70\ \text{kg}}\right)^{0.7}$ [scaling of 720 mg dose used for liquid formulation due to lower bioavailability]).[96,239] The final average efavirenz dose used in the study using solid formulations was 14.2mg/kg/day.[96] Effectiveness and safety of efavirenz-based ART in a clinical setting was confirmed in a number of small studies (8 - 33 participants), where efavirenz dose ranged between 10.0 – 16.7 mg/kg, in children with prior exposure to ARVs in combination with NRTIs[270,271] or PIs,[272] and in treatment-naïve individuals.[273] An alternative, simplified weight band dosing was shown to provide similar virological outcome in a study by Scherpbier *et al*.[274] and has been used since.

2.3.1.7.2 Paediatric underdosing

Despite favourable virologic outcomes of efavirenz treatment a number of pharmacokinetic investigations reported underdosing in children. Van Henting *et al*.[275] showed that 63% of children receiving the recommended bodyweight adjusted dose of 10 -15mg/kg did not achieve the target exposure range of 60 – 120 mg*h/mL.[96] Suboptimal exposures were also highlighted in a number of studies with weight band dosing[56] leading to modification of guidelines in 2010[11] (Table 2.5). In a small study in South Africa Ren *et al*.[97] showed that 40% of children had efavirenz concentrations <1mg/L, 19% of children (44% weighing <15 kg) were reported to have similar low exposures in a study in Burkina Faso.[32] Sub-therapeutic (<1mg/L[94]) efavirenz concentrations following WHO 2006 recommendations[56] were also reported in high proportion of children in other South African cohorts,[34,74] in 20% of children in a study in Rwanda,[99] in 34% of trough measurements in ARROW,[41] and in 15% of trough measurements in a Thai children.[98] CHAPAS-3 was the first trial aiming to describe

and evaluate the pharmacokinetic exposure in children under new WHO 2010[11] efavirenz dosing schedule (with one modification - following previous reports the dose in the weight band 20 - 25kg was increased to 400mg – Table 2.5).

Table 2.5 Comparison of efavirenz paediatric dosing recommendations and dosing tested in CHAPAS-3

Weight band	WHO 2006	WHO 2010	CHAPAS-3
10 - 13.9 kg	200*	200	200
14 - 19.9 kg	250*	300	300
20 - 24.9 kg	300	300	400
25 - 29.9 kg	350	400	400
30 - 34.9 kg	400**	400	400
35 - 39.9 kg	400	400	400
> 40 kg	600	600	600

Note: Dosage presented in mg

*WHO 2006 weight bands 10 - <15 kg and 15 - <20kg; **Sustiva Summary Product Characteristics (2004/2008) in weight band 25 - 32.5 kg recommended efavirenz dose 350mg, 32.5 - 40 kg - 400mg

2.3.1.7.3 Paediatric pharmacokinetics of efavirenz

Until recently use of efavirenz was limited to children >3 year old, where all metabolic pathways of drug clearance are expected to be fully mature.[276] The differences between PK in adults and children are caused mainly by differences in size and are commonly accounted for using allometric scaling of body weight.[224,225] Following theory of allometry (also see Chapter 2.3.2.8.3) the weight effect on volume of distribution and clearance can be described as follows: $V_{weight} = \left(\frac{weight}{70kg}\right)^{1}$ and $CL_{weight} = \left(\frac{weight}{70kg}\right)^{0.75}$ what has been implemented in majority of paediatric pharmacokinetic models for this drug (Table 2.11 and 2.12 in Appendix to Chapter 2). In addition clearance rates in children were shown to be increased beyond adult values around 4-5 years of age,[277] which explains higher clearance reported in paediatric investigations (11.2 – 14.5 mg/L in children[32,34,40,215] vs 8 - 11.7 L/h in adults[168–170,172] at steady state scaled to 70kg). In an investigation by Saitoh *et al.*[278] clearance in younger children (3-5 years) was 47% higher than in the older group (>5 years), an effect also confirmed in studies from Burkina Faso[32] and South Africa.[97] The maturation of efavirenz metabolism was recently described by Salem *et al*.[162] using a sigmoid E_{max} model (90% of adult values by 9 months of age) confirming this effect should not present children >3 years of age. In addition the authors reported that efavirenz bioavailability of liquid formulations increases with age (90% of "full" bioavailability observed in older children in achieved by 8 years of age.

Nonetheless, similarly to adults, the high variability in efavirenz PK observed in children has been attributed predominantly to pharmacogenetic factors. Other variables previously confirmed to affect efavirenz PK in children include compliance, formulation effect (in particular liquid formulations) and drug-drug interactions.

2.3.1.7.4 Pharmacogenetics of efavirenz in children

The effect of SNP 516G>T on efavirenz PK in children was first described by Saitoh *et al*.[278] who reported an 18.5% drop in clearance for CYP2B6 516GT and 57% for 516TT. Similar effect was shown in population pharmacokinetic investigations by ter Heine *et al*.[40] and Viljoen *et al*.[97] (29.7-37.2% decrease in clearance for 516GT and 59.4-66.4% for 516TT) but not in a recent investigation by Salem *et al*.,[162] where children 516GG and 516GT had comparable clearance rates and 516TT had 51% lower values. It could be speculated that those differences originate partly due to much younger age of children in the study by Salem *et al*. (included 2 months to 3 years of age) and related age driven differences in efavirenz clearance and bioavailability, and use of liquid formulation. In addition, ter Heine *et al*.[40] and McIlleron *et al*.[74] found that the subtherapeutic efavirenz concentrations were particularly prevalent in children with 516GG genotype (having highest clearance rates).

The data on the effect of SNP 983TC in children is scarce. Mutwa *et al.*[99] reported that Rwandan children with a variant allele had average efavirenz concentrations higher than wild type (3.1 mg/L in 983TC and 1.8 mg/L in 98TT). Mixed effects linear regression was recently used in a South African study,[76] which included a small proportion of children, to confirm associations of the combined *CYP2B6* 15582C>T|516G>T|983T>C genotype and efavirenz concentrations. The effect of those SNPs on efavirenz clearance in children has however never been previously estimated.

Table 2.6 Predictors of increased risk of virological failure for efavirenz and nevirapine (part 1)

Drug	Reference (year)	PK	Covariates	Population	n	Method	VL Target [copies/mL]
EFV	Starr *et al*.[96] (1999)	Not analysed	**Uni:** (A)°°° $\log_2$ bCD4% , ↓WAZ, ↑bVL / (B)°°° ↓WAZ, ↑bVL **Multi:** (A)°°° ↓WAZ, ↑bVL / (B)°°° ↑bVL	PACTG 382 study	57	Cox	400 (A)† 50 (B)†
	Brundage *et al*.[101] (2004)	↓AUC	**Uni:** ↓IPAM, ↑bVL, ↓bCD4%, ↓WAZ **Multi:** ↓IPAM, ↑bVL, ↓AUC	PACTG 382 study	50	Cox, TSSA	400
	Hirt *et al*.[32] (2009)	↓C_{min}, ↓AUC	Not analysed	Burkina Faso	48	Fisher's exact test	300
	Fletcher *et al*.[100] (2008)	↓AUC	Not analysed	PACTG 382 study	50	Chi-square, logistic regression	400
EFV/NVP	Janssens *et al*.[279] (2007)	Not analysed	**Uni:** ↑orphan status, ↑male gender **Multi:** ↑orphan status	Cambodian	212	logistic regression	400
	Kamaya *et al*.[150] (2007)	Not analysed	**Uni:** ↑male gender, ↑ bCD4%<5%, ↑NVP **Multi:** ↑male gender, ↑bCD4%<5%, ↑NVP	Uganda	250	logistic regression	400
	Jittamala *et al*.[149] (2009)	Not analysed	**Uni:** ↑NVP (vs other treatments), ↑male gender, ↑age, ↑adherence (<95%) **Multi:** ↑NVP (vs other treatments)	Thailand	202	Cox	50
	Emmett *et al*.[280] (2010)	Not analysed	**Chi^2:** ↑NVP (vs other treatments), ↓age, ↑maladherence, ↑bCD4% <25% **Multi:** ↑maladherence, ↑bCD4% <25%	Tanzania	206	Chi-square, logistic regression	400
	Lowenthan *et al*.[151] (2013)	Not analysed	**Multi:** ↑NVP (vs other treatments)	Botswana	804	Cox	400
	Bunupuradah *et al*.[154] (2015)	Not analysed	**Multi:** ↑NVP (vs other treatments), age (↑<3 years & 10–16 years)	Thailand	840	Cox	1000

Note: ↑indicates increased risk of virological failure (with increasing values of the covariate if continuous, or presence of covariate if categorical), ↓indicates reduced risk of virological failure (with increasing values of the covariate if continuous, or presence of covariate if categorical)

††two efficacy cut-offs used: (A) 400 copies/mL, (B) 50 copies/mL; bCD4% - baseline CD4 percentage, bVL – baseline viral load, Cox – Cox proportional hazards regression, EFV – efavirenz, IPAM – integrated pharmacokinetic adherence measure, Multi – multivariate analysis, NVP – nevirapine, TSSA - tree-structured survival analysis, Uni – univariate analysis, WAZ – weight-for-age-adjusted z-score

Table 2.7 Predictors of increased risk of virological failure for efavirenz and nevirapine (part 2)

Drug	Reference (year)	PK	Covariates	Population	n	Method	VL Target [copies/mL]
NVP	Musoke *et al*.[61] (2015)	Not analysed	**Multi:** ↑male gender, ↓adherence, ↑bVL, ↑syrup formulation	Uganda/ Zimbabwe	367	logistic regression	80
EFV/NVP /PI	Duong *et al*.[153] (2014)	Not analyses	**Multi:** ↑NVP (vs other treatments), ↑age, ↑pMTCT, ↑ART start <2004, ↑bVL*	UK	997	Poisson mixed models	400
	Mgelea *et al*.[281] (2014)	Not analyses	**Multi:** ↑NVP (vs other treatments)	Tanzania	218	Logistic regression	400
NVP/PI	Violari *et al*.[145] (2012)	Not analysed	**Multi:** ↑NVP (vs other treatments), ↑bVL	Study P1060	288	Cox	drop >1 log10 from baseline
	Lindsay *et al*.[282] (2014)	Not analysed	**Multi:** ↑NVP (vs other treatments), ↓bCD4%, ↑bVL	Study P1060	120	Cox	400**

Note: ↑indicates increased risk of virological failure (with increasing values of the covariate if continuous, or presence of covariate if categorical), ↓indicates reduced risk of virological failure (with increasing values of the covariate if continuous, or presence of covariate if categorical)

*at 12 months only, **composite end point: drop >1 log10 from baseline at 12 months or VL>400 copies/mL at month 24; ART – antiretroviral treatment, bCD4% - baseline CD4 percentage, bVL – baseline viral load, Cox – Cox proportional hazards regression, EFV – efavirenz, Multi – multivariate analysis, NVP – nevirapine, PI – protease inhibitor, pMTCT – prevention of mother to child transmission

2.3.1.7.5 Paediatric PK/PD relationship and therapeutic targets

The PK/PD relationship in children has been little studied and the adult efficacy cut-off of 1mg/L[94] is customarily used, even though it was recently questioned whether this cut-off can be universally extrapolated between populations and age groups.[72] The first target exposure range suggested in children was based on AUCs observed in the adult phase 2 study (efavirenz dosed 600 mg once daily) and was derived based on 50th to twice 50th percentile of those values (60 - 120 mg•h/mL).[96] The PK/PD analysis of data from PACTG 382 conducted by Brundage *et al.*[101] showed that children with efavirenz AUC <60 mg•h/mL were at higher risk of treatment failure. The risk was further modified by baseline VL and treatment adherence expressed as integrated pharmacokinetic adherence measure (IPAM) calculated based on the intra-individual variability between PK measurements. The data from PACTG 382 was re-analysed by Fletcher *et al.*[100] providing a more thorough investigation into the relationship between systemic exposures to efavirenz and virological outcome in children suggesting a new efficacy threshold of AUC > 49 mg•h/mL based (cut-off relating to 1st quartile of observed AUC). An alternative threshold (of 1.1 mg/L) was suggested by Hirt *et al.*[32] (Table 2.4). Both those cut-offs were derived from small cohorts of children using simplistic methods based on comparison of a priori defined break points and none of them attempted to identify predictors of virological outcome other than the observed drug exposures. No association between efavirenz mid-dose concentrations and virological response was detected in a study in Rwandan children by Mutwa *et al*.,[99] which can be attributed to a sample size. A novel approach to PK/PD data analysis and establishing therapeutic targets was recently suggested by Bouzza *et al*.,[240] who by applying an HIV dynamics model derived an EC_{90} of 3.3 mg/L for efavirenz and suggested that if used in a triple combination with lamivudine and didanosine it contributes towards 65% of observed total treatment antiretroviral effect.

All other paediatric investigations evaluating the effectiveness of efavirenz treatment explored associations between a selection of factors independently of drug concentrations and showed inconclusive findings across studies (Tables 2.6 and 2.7).[99,100,149–151,279] Virological failure was associated with factors previously found in adults - lower adherence,[101,149] higher baseline VL,[96,101] lower baseline CD4%,[101,150] and male sex,[149,150] as well as variables specific to this population - orphan status,[279] type of caregiver,[283] and lower weight-for-age Z-score (WAZ).[96] Noteworthy is that that the suppression rates seen in children are lower than previously reported adults (24-52% vs 80-90% achieve VL<50 copies/ml at week 48, respectively).[283]

2.3.1.7.6 Paediatric safety

Literature indicates similar differences in the safety profile of efavirenz between children and adults, maily in terms of severity and incidence of adverse events. Majority of the early clinical studies showed

that the adverse events in children were rare, transient and mostly occurring within first weeks after treatment initialisation. The incidence of neurotoxicities observed in children varied between investigations: a study in a French cohort reported CNS toxicity in 37% of participants,[271] 20% in a small German study,[273] and 16% in a large study in Ugandan children. Efavirenz associated persistent CNS side effects resulted in treatment discontinuation in 18.2% of children in a study by Wintergerst *et al*.[284] Nonetheless, more recent investigations imply that more severe and complex cases might have been previously underreported. A significant association between efavirenz concentrations >4mg/L and psychiatric side effects was reported in a Thai study,[67] and persistent high concentrations have been linked to cases of psychosis in children.[285] Severe central nervous system manifestations such as seizures, cerebellar dysfunction as well as aggressive behaviour, anti-social behaviour and poor school performance were recently associated with extremely high efavirenz concentrations (>20mg/L) in children in South Africa.[286] All of those individuals had impaired efavirenz metabolism due to *CYP2B6* polymorphisms and authors postulated that dosage optimisation based on both *CYP2B6 516G>T* and *983T>C* genotype could help prevent similar neurobehavioral or CNS abnormalities in African children. A lot remains unknown about the efavirenz CNS toxicity and its effect on brain development in children and the knowledge gaps were recently highlighted in a review by Van der Wijer *et al*.[287]

2.3.1.7.7 Paediatric dose optimisation

In addition to dose increases in certain paediatric weight bands following the aforementioned reports of under-dosing,[32,275,288] it has been documented that treatment with liquid formulations requires further dose adjustment, due to reduced bioavailability.[40,162,239] To date there have been two investigations suggesting genotype based dose optimisation in children, and neither of them accounted for the effect of SNP 983TC.

In order to prevent sub-optimal exposures in children with *CYP2B6* 516GG genotype ter Heine and co workers[40] suggested dose increases in that group - for patients >25kg carrying two variant alleles efavirenz dose should be matched with adults (600mg), 20-25kg should receive 500mg, 15-20kg – 450mg, and 13-15kg – 400mg (current guidelines advise 400mg, 300mg, 300mg and 200mg, respectively). This algorithm was never evaluated in real life.

The results of the population pharmacokinetic/pharmacogenetic analysis by Salem *et al*.[162] and the preliminary results of genotype guided dose decreases in the 1st cohort of patients in study P1070[289] stimulated development of the new guidelines for children <3 years by the Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children at the United States Department of Health and Human Services (DHHS), which account for the individual metabolic status.[134] Unlike in other investigations (Table 2.3), children were classified as extensive metabolisers (EM), if they had either

516GG or 516GT genotype, or as poor metabolisers (PM), if they had two 516TT variant alleles (Table 2.8). Additionally, the developed dosing algorithm accounted for age driven differences in efavirenz clearance detected by Salem *et al*.[162] leading to dose increases in EM. The proposed guidelines are currently tested in study P1070[290] and should give answers to feasibility and efficacy of genotype based dose adjustment strategies in children. Their application is nevertheless limited – firstly they should not be extrapolated to children > 3 years of age, secondly, not only they don't account for differences in clearance rates between individuals carrying two version of common allele for 516G>T and individuals carrying one variant allele, but also don't take into consideration the effect of SNP 983T>C.

Table 2.8 Proposed American efavirenz treatment guidelines for children <3 years accounting for CYP2B6 516G>T genotype

P1070*			Modified FDA/EMA guidelines**	
Weight band	EM	PM	Weight band	No genotype
3 - 5	200 mg	50 mg	3.5 - 5	100 mg
5 - 7	300 mg	50 mg	5 - 7.5	150 mg
7 - 14	400 mg	100 mg	7.5 - 15	200 mg
14 - 17	500 mg	150 mg	15 - 20	250 mg
>17	600 mg	150 mg	20 - 25	300 mg
			25 - 32.5	350 mg
			32.5 - 40	400 mg
			> 40	600 mg

*genotype guided dosage currently tested in the study P1070.[290] **The Sustiva product label was recently extended in USA[268] and Europe. EM – extensive metaboliser = 516GG or 516GT, PM poor metaboliser = 516TT

On the contrary, the recently published population pharmacokinetic analysis that supported efavirenz label extension to children < 3 years (Table 2.8) presented conflicting results. The model by Luo *et al*.[291] showed that individuals with *CYP2B6* 516GT genotype had 24% reduced clearance and 516TT had 61% lower clearance rate, nonetheless the authors concluded that due to large variability between genotypic groups the CYP2B6 genetic status was not predictive of exposure and was not informative in guiding paediatric dosing. The conclusions drawn by authors are discussed in Chapter 8.4.

2.3.2 Nevirapine

2.3.2.1 Pharmacokinetics

According to manufacturer's information after repeated administration of twice daily 200mg dose in adults nevirapine has a steady state C_{max} = 5.74 mg/L (5.00 – 7.44), which is reached 4 hours after administration, C_{min} = 3.73 mg/L (3.20 – 5.08), and AUC = 108 mg·h/L (96.0 – 143.5).[292] Nevirapine concentrations increase linearly with dose in the licensed range of 200 - 400 mg. Oral bioavailability is approximately 90% and is comparable for solid and liquid formulations.[293] Manufacturer advises that food does not affect nevirapine pharmacokinetics, nonetheless one study reported increased bioavailability when the drug was ingested with food, and another that food increased its absorption time.[294] Nevirapine is lipophilic (it is essentially non-ionized at physiologic pH), in plasma it is approximately 60% protein bound, it crosses the placenta, the blood-brain barrier (concentration in cerebrospinal fluid is approx. 55% lower than in plasma) and is found in breast milk.[292] Population pharmacokinetic analyses (Tables 2.16 to 2.18 in Appendix to Chapter 2) show that nevirapine has a 1-compartmental distribution, with volume of distribution ranging between 84.5 L and 153 L[66,72,73,294–297] for average weight of 70kg (although an investigation by Chou *et al*.[298] reported 223 L for average weight of 55kg). Nevirapine ka has been estimated to range between 1.25 h^{-1} and 1.67 h^{-1} [66,73,294,296–298] (with exception of Dickinson *et al.*[72] – 0.578 h^{-1}), and oral clearance between 2.95 L/h and 3.49 L/h[66,72,294–298] (both processes were described using 1st order kinetics). Similar to efavirenz delays in nevirapine absorption were described through transit compartments.[294]

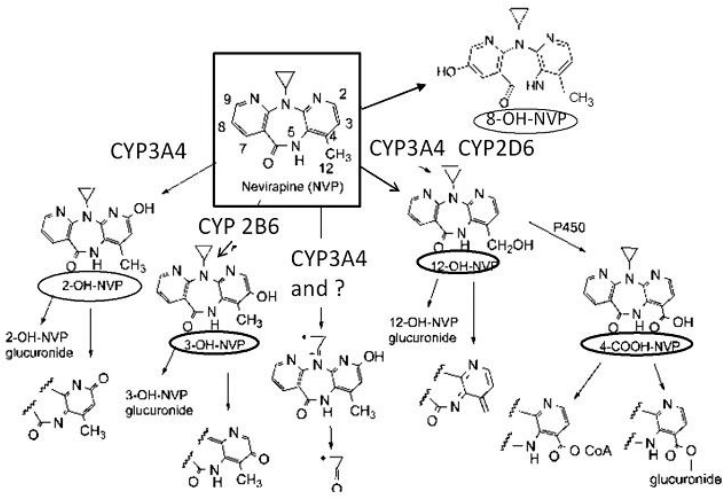

Figure 2.6 Suggested metabolic pathways for nevirapine with corresponding cytochrome P450 catabolic enzymes and main metabolites (from Fan-Havard et al.[65])[G]

[G] Reprinted from the *AAC*, 57, 2154–2160, Fan-Havard, P. *et al*., Pharmacokinetics of phase I nevirapine metabolites following a single dose and at steady state. Page No. 2155, Copyright (2013) with permission from American Society for Microbiology.

Biotransformation of nevirapine is mediated though cytochrome P450 (CYP450) system and includes first step hydroxylation catabolised though an number of pathways including primarily CYP3A4, CYP2B6, CYP2D6, followed by UGT catabolised glucuronidation (Figure 2.6).[65,292,299] Similar to efavirenz, nevirapine clearance rate increases after repeated dosing (by 23% - 39%[66,300]) leading to significant reduction of its half-life (from 45h to 25-30h).[292] Recent investigation identified different contribution of metabolic pathways after single dose and at steady state.[65] The main metabolite identified after administration of a single nevirapine dose is 12-OH-NVP with the corresponding metabolic pathway being CYP3A4.[301] The metabolic index (ratio of the metabolite AUC to the nevirapine AUC) for 12-OH-NVP after repeated dosing remains unchanged, but decreasing 2-OH-NVP concentrations suggest nevirapine marginally inactivates CYP3A enzymes (Figure 2.6).[65] On the contrary, repeated dosing causes substantial induction of CYP2B6 pathway (and increased concentrations of the corresponding metabolite - 3-OH-NVP). CYP2B6 activation is the main mechanism for the observed auto-induction in nevirapine clearance.[65] In addition, nevirapine has been shown to significantly induce the P-gp expression.[216]

Low apparent nevirapine clearance suggests that biotransformation occurs mainly in the liver and that the intestinal first-pass effect is negligible.[65] Nevirapine is excreted primarily in form of glucuronide conjugates of hydroxylated metabolites in the urine.[292] Study in rats showed that nevirapine undergoes enterohepatic recycling,[302] and although a second peak was seen in some of the early studies in adults,[91,295] it is an effect not observed in all of the patients.[50] Despite a long half-life allowing for a once daily dosage nevirapine in adults is administered twice a day (200 mg) due to concerns about high peak and low trough concentrations observed under once daily administration (Chapters 2.3.2.5 - 2.3.2.7).[303]

2.3.2.2 Pharmacogenetics

2.3.2.2.1 CYP2B6

Similarly to efavirenz nevirapine PK exhibits high levels of between subject variability, which has been similarly associated with highly polymorphic CYP2B6 metabolic pathway.[43,186,278,304] Due to a higher contribution of CYP3A to biotransformation of nevirapine (Figure 2.6) the effect of CYP2B6 polymorphisms on its pharmacokinetics is not as strong as observed for efavirenz.[186,278] Rotger *et al.*[186] showed that the mean plasma efavirenz AUC for individuals with 516GG genotype were 3-fold higher than in individuals homozygous for common allele, whereas the difference was 1.7-fold for nevirapine. Similar association were replicated in a small cohort of 23 Ugandan patients, where the mean ratio of trough concentrations for 516TT vs. 516GG individuals was 1.51.[304] Population pharmacokinetic

studies estimated that the average steady state clearance of nevirapine is reduced by 9-19.4%, if one variant allele is present, and 23-37% for two variant alleles.[72,73,298,300,305]

In addition to SNP 516G>T nevirapine pharmacokinetics is further modified by the effect of 983T>C. This association was first detected by Wayne *et al*.[198] in patients from the German Competence Network for HIV/AIDS. Noteworthy, the effect of SNP 983T>C on nevirapine clearance is more prominent than previously detected for efavirenz. A population pharmacokinetic investigation by Schipani *et al.*[73] showed that in the presence of one variant allele for 516G>T nevirapine clearance is reduced by 14%, 37% for two variant alleles, whereas in individuals wild type homozygous for 516G>T but with one variant allele for 983T>C the observed reduction was 40%. This associating was replicated in a large Malawian study where the apparent nevirapine clearance was reduced by 23% in patients with CYP2B6 983TT|516TT, and by 36% 983TC|516GG or GT, compared to the reference 983TT|516GG.[72] These associations were recently also replicated in study in Zimbabwean patients.[306]

A large pharmacogenetic investigation by Haas *et al.*[64] evaluating associations between single dose nevirapine PK and 51 different CYP2B6, ABCB1, CYP3A4, and CYP3A5 polymorphisms did not show any significant effect of combined 516G>T|983T>C genotype. This surprising lack of effect could be linked to the aforementioned differences in the contribution of CYP2B6 pathway to its total clearance under single- and multiple-dose conditions.[65] This also is the only study that evaluated the composite effect of those SNPs in terms of a metabolic subgroups (proposed as "extensive metabolizer" [EM] denoted no variant allele at either position 516 or 983; "intermediate metabolizer" [IM], a single variant allele at either position 516 or 983, but not both; "slow metabolizer" [SM], 2 variant alleles [i.e., either 516TT, 983 CC, or 516 GT with 983 TC]).[64]

2.3.2.2.2 CYP3A4/5

CYP3A is known to be less polymorphic than CYP2B6, but it has been shown to be affected by SNP *CYP3A5* 6986 G>A.[307] The CYP3A5*3 allele (G at position 6986) creates a cryptic splice site, linked to aberrant mRNA with a premature stop codon. Individuals with at least one A allele (CYP3A5*1) produce high levels of full-length CYP3A5 mRNA and express an active CYP3A5 enzyme, while those carrying the CYP3A5 6986GG (CYP3A5*3) genotype have very low (or even undetectable) hepatic CYP3A5 protein content.[308] Due to substantial contribution of CYP3A5 pathways to nevirapine biotransformation it has been speculated that its PK could be affected by SNP *CYP3A5* 6986 G>A, which has been shown to alter clearance of other CYP3A5 substrates.[87,88] The frequency of *CYP3A5* 6986G allele differs between populations and has been reported to range from 0.87 – 0.94 in Caucasians,[69,309] 0.65 in Cambodians,[298] 0.74 – 0.78 in Asians,[309] and 0.36 in black Africans.[69] Despite having been tested in a number of pharmacogenetic nevirapine investigations this association was reported to be

significant only in one study in Malawian patients (CYP3A5*3 decreased navirapine AUC by 31%[310]). All other studies revealed no association.[72,300,304,311,312] Some focus has been contributed to SNP CYP3A4*1B (329A>G) but this association has similarly not been confirmed in any of the studies.[300,304,310–312] Moreover, reports in other CYP3A4/5 substrates revealed haplotype CYP3A4*22 as a promising new target.[85,307,308]

In addition, nevirapine clearance has been reported to be affected by SNP *CYP2C19 8402G>A* (26.8% reduction reported in re-analysed data from 2NN study),[300] however significance of this association has not been replicated and nevirapine is not known to by metabolised by CYP2C19 pathway. A recent analysis in a Cambodian cohort did not confirm this finding but revealed two two novel SNPs in CYP2B6 (rs7251950 and rs2279343) which were highly correlated with nevirapine concentrations.[311 312]

2.3.2.2.3 Nuclear receptors

The information on the effect of polymorphisms in genes coding nuclear receptors for nevirapine is scarce, but a study by *Faucette et al*.[313] showed that nevirapine was a CAR inducer which was hypothesised to be linked with the auto-induction of its clearance.[314] To date two studies explored the associations between polymorphisms in NR1|2 and NR1|3 but both did not find any significant associations.[310,311]

2.3.2.2.4 Transporters

The effect of polymorphisms in genes coding membrane transporters for nevirapine remains unclear and controversial. Störmer *et al*.[216] in an *in vivo* experiment showed that neither efavirenz, nor nevirapine were substrates for P-gp, but nevirapine caused an up to 3.5-fold concentration-dependant induction of P-gp expression.[216] The inducing effect of nevirapine on P-gp activity was confirmed in another study.[315] An association between nevirapine intracellular concentrations and P-gp expression was shown in a small study by Almond *et al.*[316] SNP ABCB1 3435C>T has been associated with the risk of hepatotoxicity in patients on nevirapine in a study by Ritchie *et al.*[317] In another investigation Saitoh *et al.*[278] found that recessive ABCB1 3435C>T hetero- or homo-zygosity were linked to increased ratio of nevirapine CNF/plasma concentrations (0.62 vs 0.43 for wild type). All other investigations did not find any significant association for any of the ABCB1 polymorphisms with nevirapine PK.[278,298,304,311,312,318]

2.3.2.3 Circadian variation

The extensive pharmacokinetic investigation by van Heeswijk *et al*.[91] reported differences between morning and evening nevirapine PK parameters in a cohort of 20 adults (dosed 200 mg twice a day). The reported differences were of small magnitude and mostly not statistically significant, which could

be attributed to the small sample size in the study. Similar diurnal variability in pharmacokinetics (although of greater magnitude) was previously reported in another group of ARV agents, protease inhibitors (PIs).[319–322] The differences in morning and evening trough concentrations vary depending on the drug and the investigation, and range between 57% and 2.5-fold, which led to suggestions of adjustment of dosing interval for some PIs (shortening of the day-time interval) to ensure more balanced exposures over the 24h cycle.[321,322] The observed variation was linked to differences in hepatic blood flow over the 24h cycle.[323]

The diurnal pattern for nevirapine pharmacokinetics was characterised in a population pharmacokinetic analysis by Elsherbiny *et al.*[50] The variability was described using a step function related to the day-night cycle (day was defined as the time between sunrise and sunset, while night was the time between sunset and sunrise) included in the model on ka and CL. Nevirapine CL was 27% higher during the night and ka was 10-fold lower. The observed differences in absorption were explained though faster gastric emptying and a higher perfusion of the gastrointestinal tract in the mornings leading to rapid absorption by passive diffusion observed in other drugs.[324] It was hypothesised that the rapid absorption in the morning may decrease the time of contact between nevirapine and the metabolizing enzymes in the gut, thus resulting in decreased first-pass metabolism and increased bioavailability of nevirapine, leading in consequence to the observed lower CL/F1 in the morning (modelled as higher clearance during the night). Despite a theoretical plausibility of this hypothesis it is not in line with more recent findings characterising a different pattern of circadian oscillation in CYP4A4 activity.[325]

This effect was extensively studied in a CYP3A4 probe – midazolam. Population pharmacokinetic investigation by Tomalik-Scharte *et al*.[89] characterised the observed daily oscillation in CYP3A4 activity using a cosine function with peak clearance values around 3pm and nadir around 3am. Van Rongen *et al*.[90] presented similar findings (peak oscillation in clearance around 7pm), additionally reporting circadian pattern in oral bioavailability with a peak around midday and ka with a peak at 2pm. It could be speculated that due to a high contribution of CYP3A4/5 enzymes to nevirapine metabolism a similar circadian oscillation could be observed in its clearance.

The molecular mechanism for the observed rhythmicity in CYP3A4 has been linked to the oscillation in the expression of CYP3A4 mRNA in human hepatic cells, which is regulated by a circadian expression of two transcriptional factors (D-site-binding protein and E4 promoter-binding protein 4).[325] The expression of those factors is directly controlled by a particular gene referred to as the CLOCK gene (*Circadian Locomotor Output Cycles Kaput*) generating circadian oscillations contribution to the observed pattern.[326]

2.3.2.4 Other predictors of variability in the nevirapine pharmacokinetics

In addition to the above mentioned factors the pharmacokinetics of nevirapine has been previously shown to be affected by a sex, race, hepatitis B co-infection, selection of laboratory markers and interactions with other drugs. The clearance of nevirapine was reported to be lower in females in 2 studies - it was 23% lower in an investigation by Zhou *et al*.[295] and 14% lower in 2NN study.[66] Similar to efavirenz, the race effect on nevirapine clearance detected in some studies[66,296] was related to the differences in the distribution of *CYP2B6* polymorphisms between populations. Additionally, De Maat *et al.*[296] reported that nevirapine clearance was reduced by over 50% in patients with hepatitis C co-infection. Clearance was also lower in patients with increased ASAT levels,[296] and linearly correlated with increasing plasma albumin levels.[50]

The interaction between nevirapine and rifampicin is hypothesised to have a dual mechanism. Firstly, rifampicin is a potent inducer of CYP3A family in the liver and intestinal wall, secondly, it also induces the expression of transporter proteins such as P-gp.[327] It is currently known that both of those pathways are linked to rifampicin-induced up-regulation of nuclear receptors.[328–330] Several investigations showed that rifampicin co-treatment significantly reduced the nevirapine levels (up to 55% lower) exposing patients to increased risk of sub-therapeutic concentrations,[331,332] and modelling analyses identified this effect as changes in nevirapine clearance (37% increase during co-treatment[50]) or bioavailability (39% reduction[294]). It was previously suggested that during rifampicin co-treatment dose of nevirapine should be increased from 200mg to 300mg to prevent the risk of virological failure related to sub-optimal drug exposures.[50] The current guidelines suggest that HIV-infected patients who contract TB should be switched from nevirapine based regiment to efavirenz.[6]

Other important interactions include protease inhibitors, in particular ritonavir (casing increase of nevirapine concentrations through inhibition of CYP3A pathway),[301] and zidovudine (nevirapine was also shown to reduce the bioavailability of zidovudine by 23% with no effect of zidovudine on nevirapine PK).[295]

2.3.2.5 PK/PD relationship and therapeutic targets

The current therapeutic target for nevirapine was set to 3 – 8 mg/L[95,333] and similarly to other ARVs is defined by lowest concentration providing optimal virologic response and highest concentration not causing any safety signals. The associations of the concentration thresholds for nevirapine are weaker than for efavirenz and their application has been previously questioned[234] (in particular the upper therapeutic target[105]). Furthermore, the predictive power of those targets was never previously evaluated in children, or in black African patients in a resource limited setting, which raised doubts about their universal application across populations.[72]

The results of the initial single-rising-dose study in nevirapine concluded that a daily dose of 12.5mg would be sufficient to inhibit replication of wild-type HIV-1 in human T-cells, but that it was well tolerated in doses up to 400mg.[334] Results of further investigations indicated that only a dose of 400mg would provide a sustained virological suppression,[335,336] and additionally, in order to prevent emergence of resistant HIV strains the drug should be administered in combination with 2 NRTIs, and not as a stand alone treatment.[335,337,338] Paradoxally however, based on the results of those studies, it was believed that a relationship between pharmacological exposure and virological effect for nevirapine did not exist (because the plasma concentrations necessarily for achieving positive treatment effect were several fold higher than the in vitro concentrations giving 90% inhibition [IC90] for wild type virus).[339]

Table 2.9 Previously published nevirapine therapeutic targets

Pop	Ref (Year)	Target	Method	VL [copies/mL]	n
Adults	Veldkamp *et al.*[340] *(*2001)	C_{TDM} > 3.5 mg/L	Pre-defined - median concentration plus logistic regression	20	51
	de Vries-Sluijs *et al.*[341] (2003)	C_{TDM}= 3.0 – 8 mg/L	Pre-defined - selected based on study by van Heeswijk *et al*.[91] plus Cox multivariate models	500	189
	Gonzalez de Requena *et al.*[342] (2005)*	C_{TDM} > 4.3 mg/L	Receiver operator curve (ROC)	50	178
	Duong *et al.*[343] (2005) **	C_{TDM} > 3.4 mg/L	Logistic regression applied to cut-off by Veldkamp *et al.*[340]	20	74

*patients ART-naïve, experienced and switching from PI-based regiment; **patients switching from PI-based regiment.

The efficacy targets for nevirapine suggested in the literature are presented in Table 2.9. The first target was derived in the PK/PD investigation of INCAS trial, where individuals with higher nevirapine concentrations had better virological outcome and shorter time to initial virological suppression.[340] Average nevirapine TDM plasma concentrations above population median at multiple time points (3.45 mg/L at week 12, 3.69mg/L at week 24, 3.84 mg/L at week 36 and 3.79 mg/L at week 52) were predictive of virological suppression at week 52 and Veldkamp *et al.* suggested a cut-off of 3.5 mg/L as a potential efficacy threshold. De Vries-Sluijs *et al*.[341] verified the association between the high

nevirapine exposures and the virological suppression and proposed a new cut-off of 3 mg/L. The authors detected that the risk of virological failure was further increased by younger age, Black race, high initial HIV-RNA levels and a low CD4+ cell count and the calendar year of ART start (all significant at p<0.1). Those association were not confirmed in the 2NN study by Van Leth *et al.*,[234] where the analysis was limited to treatment adherent patients only (>95% adherence) and no significant predictors of virological outcome could be found, or in fact any concentration-response relationship for nevirapine.

The presented studies were conducted in patients with no prior exposure to ART at the time of enrolment. A study by Gonzalez de Requena *et al.*[342] included a mixture of ART-naïve and experienced individuals and found a threshold of 4.3 mg/L as most predictive of virological failure. The risk was significantly higher in ARV-experienced patients (in particular ones with detectable VL at baseline). Lastly, the 3.4 mg/L (suggested by Veldkamp *et al*.[340]) was validated by Duong *et al.*[343] on data in ART-experienced patients switching to nevirapine from a PI-based regimen. In that study patients who retained virological suppression had significantly higher nevirapine concentrations than ones who experienced virological failure (4.6 mg/L vs. 2.6 mg/L, respectively).

2.3.2.6 Safety

Treatment with nevirapine has been associated with development of hypersensitivity reactions and hepatotoxicity with frequency of those events differing between studies and populations. An early open-label phase I/II study by Havlir *et al.*[336] reported that 48% of patients developed rash shortly after treatment initiation. Nevirapine in this study was dosed once daily as 400mg with no 200mg lead in period. A safety review by Pollard *et al*.[344] of early nevirapine studies (including 906 adult and 468 paediatric patients) revealed that rash was the most common adverse event (16% of patients, vast majority developed it within the first 6 week since treatment start) and suggested dose escalation as a preventative measure. Effectiveness of this approach was confirmed in a study by de Barreiro *et al*.,[345] where incidence of rash under standard treatment approach (no dose escalation) was halved after implementation of alternative approaches (from 18.7% to 11.2% on dose escalation and 7.7% on dose escalation plus oral during the first 2 weeks of treatment). In contrast, a 2-fold higher incidence of rash was associated by de Maat *et al*.[346] with nevirapine plasma concentrations > 5.3 mg/L.

Contradictory findings were presented in the analysis of 2NN study by Kappelhoff *et al*.[105] who reported no significant association between nevirapine concentrations and adverse events, although the incidence was higher in females, patients with a higher CD4 cell count at baseline, patients from Thailand and ones with hepatitis co-infection. No association between nevirapine exposures and hypersensitivity was similarly found by Dickinson *et al*.[72] in Malawian patients. A review by Cooper *et*

al.[303] showed that despite those contradictory reports the incidence of rash was higher among individuals dosed with nevirapine 400mg once a day and to prevent the excess toxicities its daily dose is currently split (2 x 200mg). Recent studies showed that the mechanism for the nevirapine induced hypersensitivity reactions is idiosyncratic and immune-mediated[347] and was associated with the 12-OH metabolite.[348] Most recent investigations indicate that higher susceptibility to developing nevirapine related hypersensitivity has a genetic background.[349,350]

The safety review by Pollard *et al*.[344] showed additionally that 1% of patients in nevirapine trials developed hepatitis. Gonzalez de Raquena *et al*.[351] reported that high nevirapine concentrations (> 6mg/L) and hepatitis C co-infection were risk factors for developing of liver toxicity. Another study by the same group indicated that nevirapine concentrations were associated with transaminase elevations in both HEP-C+ and HEP-C- patients.[352] In addition, Sanne *et al*.[353] identified body-mass index (BMI) <18.5, female sex, serum albumin level <35 g/L, mean corpuscular volume >85 fL, plasma HIV-1 RNA load <20,000 copies/mL, aspartate aminotransferase level <75 IU/L, and lactate dehydrogenase level <164 IU/L as independent risk factors for development of severe hepatotoxicity in patients treated with nevirapine, but did not evaluate the association with actual drug concentrations. The authors concluded that the use of nevirapine in female patients with a low BMI should be discouraged. No association between nevirapine and hepatotoxic events was found in a number of other studies,[104,105,354–358] including a large meta-analysis by Stern et al.[355] (based 8711 patients). However, it has been hypothesised that development of skin rash after start of nevirapine treatment might frequently prevent developing hepatotoxicity,[354] as it often triggers ART switch. The mechanism for the nevirapine induced hepatotoxicity is speculated to similar to hypersensitivity. It's similarly idosynchractic and related to formation of quinone methide by sulfation of 12-OH metabolite followed by loss of sulphate by P450 in the liver; increased susceptibility to hepatotoxicity was also associated with certain genetic polymorphisms.[106,299,317,347–349,359,360]

Of note is that the side effects associated with nevirapine treatment are reported to be less common in resource limited setting.[72,361]

2.3.2.7 Optimisation of nevirapine dosage

The treatment optimisation strategies for nevirapine are limited in comparison to efavirenz. Despite comparable pharmacokinetics and treatment efficacy under daily and twice daily dosing regiments, the aforementioned review by Cooper *et al*.[303] showed that once a day dosing with 400 mg was associated with a less favourable safety profile, leading to the current twice a day dosing schedule for nevirapine. Of concern are also reports of sub-therapeutic concentrations during rifampicin-based TB

treatment,[331,332] and it is recommended that in that period nevirapine is either substituted by a different drug, or its dose is increased. [50]

No previous investigation suggested genotype-guided dose optimisation strategy for nevirapine.

2.3.2.8 Paediatric investigations

To date there are few investigations describing the PK of nevirapine in children and no prior paediatric investigation explored the association between the systemic exposure to nevirapine and the virological outcome or side effects. The vast majority of paediatric investigations focus on treatment efficacy and/or safety and tolerability, primarily in comparison with efavirenz (Tables 2.6 and 2.7). The overview of the paediatric studies in nevirapine is presented below (paediatric population pharmacokinetic analyses are additionally summarised in Table 2.16 in Appendix to Chapter 2).

2.3.2.8.1 Early studies

Initial information on nevirapine pharmacokinetics, efficacy and safety in children was generated in trails ACTG 241 and 245.[362–364] The early studies were conducted before introduction of triple therapy, when only limited options were available for treatment of HIV in children (zidovudine, didanosine and lamivudine). Those studies showed that nevirapine mono- and bi-therapy (in combination with zidovudine), despite providing initial reduction in viral load, did not ensure lasting virological suppression, which led to resistant viral strains and loss of antiviral activity.[362] Much better virological outcome and durable suppression was obtained through treatment in triple combination with zidovudine and didanosine.[363] Following reports in adults, treatment in children was initiated with dose escalation, and following reports of higher clearance rates in young children, the dose in individuals ≤8 years was higher than in older children.[362] The ACTG studies utilised a mg/body surface area (BSA) dosage algorithm (120 mg/m^2 lead-in phase followed by 200 mg/m^2 every 12h in children ≤8 years and 120 mg/m^2 every 12h in children >8 years thereafter).[362] A simplified mg/kg dosing was suggested by the FDA: 4 mg/kg once daily for 2 weeks in all children followed by 7 mg/kg twice daily thereafter in children 3 months to 8 years and 4 mg/kg twice daily in children ≥8 years.[365]

The early paediatric pharmacokinetic studies, while limited to small numbers of patients, reported that the paediatric mg/m^2 (or kg) dosage provided nevirapine exposures similar to observed in adults (after administration of 200 mg twice a day).[365] Unlike efavirenz, the liquid formulations of nevirapine had similar bioavailability to its solid ones and did not require a dose adjustment.[365] In addition nevirapine was the recommended drug used in prevention of HIV mother to child transmission.[365]

2.3.2.8.2 Paediatric underdosing

Prior to availability of the paediatric nevirapine solid formulations, in order to reduce the cost of ART, increase its convenience, feasibility and coverage a number of national formularies in resource-limited countries introduced the option of treating children with adult FDC tablets. The first paediatric PK study evaluating nevirapine concentrations obtained under administration of divided adult FDC tablets reported adequate drug exposures in 34 Thai children.[366] Based on the satisfactory virological response in this study it was concluded it was an acceptable treatment option while waiting for the development of paediatric solid forumations.[366] However, similar studies in Malawian and Zambian children found that even though the exposures were adequate in older children, a high proportion of youngest children (treated with a quarter of the adult tablet) had sub-therapeutic concentrations.[77,367–370] Those studies postulated increase of the mg/m^2 dosage in the youngest/lightest children.

Anticipating the development of the paediatric all-in-one FDC tablet containing nevirapine WHO suggested in 2006[56] a new simplified weight band dosage recommendations adjusted for more aggressive dosing in the younger age groups (Table 2.10). Alternative, recommendations were proposed in a number of national formularies.[305,366,370,371]

Table 2.10 Nevirapine paediatric dosage recommendations

Weight band	WHO 2006[56]/2010[11]
3 – 5.9 kg	50
6 – 9.9 kg	75
10 - 13.9 kg	100
14 - 19.9 kg	125
20 - 24.9 kg	150
25 - 40 kg	200

Note: Dosage presented in mg under twice a day administration following a 2 week once a day lead-in phase. Children in each weight band anticipated to have a target dose of 160 – 200 mg/m^2.

The first study evaluating nevirapine exposures under the new paediatric nevirapine FDC (Triomune Baby/Junior) and WHO 2006 weight-bands was CHAPAS-1. The PK investigation in 65 Zambian children showed that even though the overall observed drug exposures were higher than in adults and above therapeutic target of 3mg/L in majority of patients, the concentrations were much more variable and sub-optimal in 6% of paticipants.[33] The proportion of individuals with low nevirapine concentrations did not differ between the higher weight bands, but was noticibly higher in children weighing < 6kg (5% vs 27%, respectively). A subsequent analysis by Filekes *et al*.[37] (after recruiting additional patients

≤ 6kg) showed that the nevirapine concentrations were on average 20% lower in that weight band (with a significantly higher proportion of sub-therapeutic exposures) compared to children weighing ≥6kg. Similar results were found in a small Indian study, where a substantial proportion of participants (35%) had subtherapeutic nevirapine trough levels (<3 mg/L), and this was more pronounced in young and stunted children.[372] Filekes *et al*.[37] concluded that the new WHO simplified weight-band dosing provides on average adequate exposures and the subtherapeutic nevirapine concentrations observed in the youngest children can be attributed to the high intra-individual variability. The given fast growth rates after starting ART (such that most infants spend only a short time in the lowest weight band) limiting the exposure to low nevirapine concentrations observed in some patients the dose in the lowest weight band should not be further increased.

Similar investigations were conducted with an alternative FDC formulation developed and used in Thailand (GPO-VIR S7),[371,373] and showed that the new paediatric tablets dosed according to alternative Thai weight-band dosing provided adequate exposures in children. In order to account for the differences in dosing schedules and formulations used in different trials a pooled population pharmacokinetic analysis including majority of previous nevirapine PK studies was conducted (PACTG studies: 245, 356, 366, 377, 403, 1056, and 1069 and CHAPAS-1 - total of 639 children).[305] Its main objective was to assess WHO simplified weight-band dosing recommendations and showed that it provided adequate drug exposures. In contrast, a much smaller analysis (95 children) by Foissac *et al*.[374] reported that while patients weighing more than 10 kg had less than 6% probability of nevirapine concentrations <3 mg/L, the probabilities were 19% and 11% in the 3–6 and 6–10 kg weight ranges, respectively. Authors suggested the doses for the 3–6 and 6–10 kg weight ranges should be increased to 75 and 100 mg twice daily, respectively.

2.3.2.8.3 Paediatric pharmacokinetics of nevirapine

Similar to efavirenz the increased clearance levels observed in nevirapine studies in younger children could be explained through the theory of allometry (Chapter 2.3.1.7.3).[224,225] Figure 2.7 presents the differences in relative clearance compared to when it's scaled linearly and in an allometric manner. It shows that actual clearance values in younger children (modelled best using an allometric power or a BSA model) exceed the values predicted through linear extrapolation. The clearance values reported in paediatric studies range between 3.93 and 5.67 L/h[278,305,366,374] in contrast to 2.95 - 3.49 L/h[66,72,294–298] reported in adults. This explains why children ≤8 years require a higher mg/kg dose than older children and adults to ensure comparable nevirapine exposures.

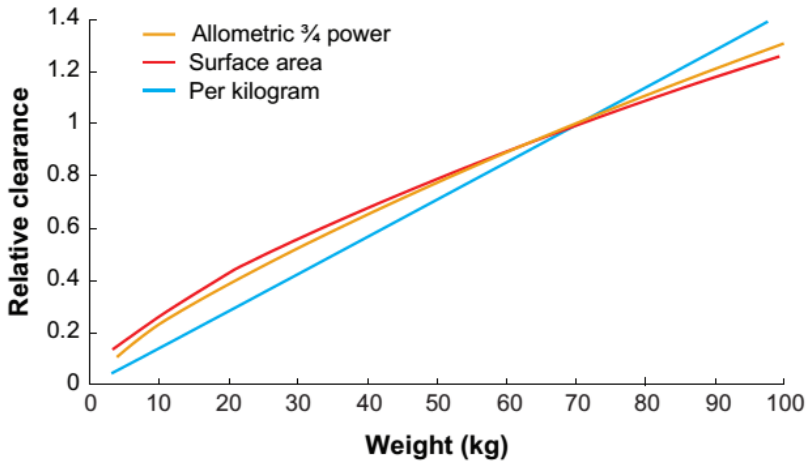

Figure 2.7 Comparison of models describing changes in relative clearance over the rage of human weight (from Anderson et al.[225])[H]

Note: A 70-kg person has a normalized clearance of 1 for each model.

Additional changes in clearance values observed in very young children can be attributed to the maturation of metabolic pathways. Nikanjam *et al*.[305] described maturation of nevirapine clearance in children using an inverse exponential function with an intercept (the clearance at birth was 39% of "mature" clearance, the "half-life" of the maturation process was 3.2 months and 100% was reached by the age of 4 years). Foissac *et al*.[374] contributed those changes to age-driven differences in oral bioavailability expressed through an E_{max} model with 50% of F1 (PMA_{50}) reached by about 6 months of life and 90% of "full bioavailability" by 4 years.

Similarly to efavirenz and presented adult nevirapine investigations, the variability in nevirapine PK in children has been primarily attributed to differences in clearance rates determined by patient's *CYP2B6* genotype. Nevirapine PK in children has been previously reported to be further affected by nutritional status (stunting and wasting) and effect of different formulations.

The effect of malnutrition was first reported in a study in Malawian children by Ellis *et al*.[77] who associated lower nevirapine exposures with stunting (lower height-for-age score) and lack of wasting (here higher BMI-for-age scores). The authors hypothesised that malnourished children have a weight lower than normal with values otherwise corresponding to higher clearance (higher clearance in younger/lighter children). Those associations were not replicated in another study in a similar population by Pollock *et al*.[368] On the contrary, Swaminathan *et al*.[372] reported that in stunted Indian children nevirapine 2h concentrations were significantly lower compared with non-stunted children,

[H] Reprinted from the *Annu. Rev. Pharmacol. Toxicol*., 48, 303–32, Anderson, B. J. *et al*., Mechanism-based concepts of size and maturity in pharmacokinetics. Page No. 310, Copyright (2008) with permission from Annual Reviews.

but there were no significant differences in trough concentrations between different nutritional groups. Furthermore, neither WAZ nor HAZ was significant in a multivariate regression after accounting for other factors.

The pooled analysis by Nikanjam *et al*.[305] reported lower bioavailability for Trioumune formulations (-42%) but the authors concluded that this effect was completely confounded by the population, as this was the only African study included in this analysis, and the detected effect could also be related to race, study design, diet, or other factors. Kashuba *et al*.[369] in a cross-over study in Malawian children reported that use of FDC tablets lead to significantly lower nevirapine exposures than liquid formulations and Vanprapar *et al*.[371] in a similar study in Thai children the opposite effect (higher exposures after administration of FDCs). In both studies this effect was small and not clinically relevant.

2.3.2.7.4 Pharmacogenetics of nevirapine in children

The information on pharmacogenetics of nevirapine in children is limited. The association between *CYP2B6* 516G>T polymorphism and nevirapine PK in children was first described by Saitoh *et al*.,[278] who reported that the clearance was reduced by 9% in the presence of one recessive allele and 31.5% if both alleles were affected. In addition, SNP *ABCB1* 3435C>T while not associated with nevirapine plasma concentrations, was predictive of concentrations in CSF. Similar associations for SNP 516G>T were replicated in a small Thai study,[373] where children with 516GT genotype had 11% higher and 516TT genotype 59% higher AUCs than wild type.

Indian children with 516TT genotype had significantly higher nevirapine exposures than 516GT or 516GG in a study by Swaminathan *et al*.[372] and the authors hypothesises that 516GT or 516GG genotype could increase the risk of developing sub-therapeutic exposures in younger and stunted children. Nikanjam *et al*.[305] reported that the clearance in poor metabolisers (516TT genotype) was 36% lower than in all other patients (combined 516GT, 516GG and missing genotype), which was comparable to a 47% reduction reported by Brown *et al*.[310] in a group of Malawian adults and children. The latter investigation also reported that the AUC was decreased by 31% in CYP3A5*3 carriers. The effect of SNP 983T>C was never previously investigated in nevirapine in children.

2.3.2.7.5 Paediatric PK/PD relationship and therapeutic targets

Based on the conducted literature review the therapeutic target for nevirapine was never previously evaluated in children. Moreover, no previous paediatric investigation described the concentration – response relationship for nevirapine (although Filekes *et al*.[37] reported no association between viral load values and nevirapine PK parameters in their study). The predictors of virological outcome in children on nevirapine were studied only in comparative investigations alongside other treatments,

mainly efavirenz-based ART but also PIs (Tables 2.6 and 2.7). An investigation in Thai children by Lapphra *et al*.[375] and Cambodian children by Janssens *et al*.[279] showed similar efficacy of nevirapine and efavirez based treatment in a clinical setting, but all other studies reported that nevirapine was inferior to other regiments.[145,149–151,153,154,280,281] In addition, replicating general findings from studies in other drugs, Kamaya *et al*.[150] reported that Ugandan children were almost twice as likely to have virological failure compared with adults on efavirenz or nevirapine based ART (26% vs. 14%; p< 0.01).

The predictors of virological failure in children on nevirapine (and efavirenz) based ART are presented in Tables 2.6 and 2.7. The majority of the studies report that the risk of virological failure increased with age at treatment initiation, for non-compliant patients and for boys. Worse virological outcome was also associated with higher baseline viral load[145,153] and lower CD4%.[150,280] A pharmacogenetic investigation by Saitoh *et al.*[278] showed that children with 516TT genotype had higher increases in CD4+ from baseline comparing to others. It could be speculated that this effect was mediated by increased nevirapine concentrations in consequence to impaired clearance in that group.

Inconclusive information exists on consequence of use of nevirapine for pMTCT on its efficacy in future life. A study in women from Botswana, who received a single dose nevirapine (sdNVP) or placebo before initiation of ART reported an increased risk of virological failure in case of prior exposure.[57] The same study followed a limited number of infants with/without sdNVP exposure reporting similar findings. A large observational study of 997 children in the UK found an increased risk of treatment failure among children with prior exposure to nevirapine for pMTCT.[153] In contrast a number of nevirapine re-use studies showed a comparable virlologic response between children who switched back to nevirapine after achieving initial suppression on a PI-based regiment and ones who stayed on the same treatment.[58,59] No effect of sdNVP exposure on the effect of nevirapine treatment was also shown in small Ugandan study,[60] as well as recently published analysis of studies P1060[145,282] and ARROW.[61]

2.3.2.7.6 Paediatric safety

Studies ACTG 241 and 245[362–364] reported that side effects related to nevirapine treatment were less common than previously observed in adults. This might be due to the fact that following previous information from adults[335,336] the paediatric patients were not started on full dose of the drug but only half dose for the first 2 weeks before up-titration. More favourable safety profile in children was confirmed in reviews of the early nevirapine studies conducted by Pollard *et al*.[344] and Bardsley-Elltiot *et al*.[365] A similar safety profile for nevirapine in children as in adults, but with a lower frequency of adverse events, was also confirmed in a study by Lepphra *et al*.[375] Treatment initiation with half-dose nevirapine was recently confirmed to be a safe tool in prevention of drug related rash in CHAPAS-1

study.[107] Nevirpaine PK was never previously associated with incidence of safety signals in children and L'homme *et al*.[33] concluded that their data suggested no effect of drug concentrations on the rate of adverse events.

2.3.2.7.7 Paediatric dose optimisation

Paediatric underdosing is of great concern for a long term success of ART, as the lack of potency in suppressing replication of the virus could lead to development of resistance mutations, in particular for drugs with a low genetic barrier to resistance such as nevirapine. In addition the data on relationship between systemic exposure to nevirapine and adverse events is inconclusive, indicating no clear relationship between concentrations and treatment safety. As a consequence a number of paediatric studies highlighted that the aim of optimisation of nevirapine treatment in children should be prevention of sub-optimal drug exposures, rather than concern about high concentrations.[33,37]

The results of studies presented in Chapter 2.3.2.8.2 contributed to the increase of recommended standard nevirapine dose with the current daily target being 300 – 400 mg/m^2 split into two parts. While it has been speculated that nevirapine pharmacokinetics exhibits diurnal variation, this effect has not been thoroughly studied. It can be assumed that due to long half-life this phenomenon is of limited significance in adults, but might be of more importance in children, where the dose cannot always be split to equal parts during the day. In addition, the studies in efavirenz show that individuals with a higher metabolic capacity determined by their *CYP2B6* 516G>T|983T>C genotype are at greater risk of developing subtherapeutic concentrations. The PK in youngest children is further modified by age-driven developmental changes and maturation. To date only two quantitative investigations aimed to characterise nevirapine paediatric PK but neither of them considered all of the aforementioned factors. Lastly, the therapeutic targets for nevirapine were never previously evaluated in children, preventing treatment optimisation in this population.

2.4 APPENDIX TO CHAPTER 2

2.4.1 Review of published population pharmacokinetic models for efavirenz and nevirapine

Table 2.11 Review of population pharmacokinetic models for efavirenz in children (part 1)

Reference (year)	Model (type, software, PK)	Covariates	Data	n	Race
Ter Heine *et al.*[40] (2008)	POP-PK(/PGx); NONMEM VI; 1-comp with 2 transit comp for absorption and 1st order elimination	**CL**: ↑ weight[a], *CYP2B6 516G>T* (GT ↓29.7%, TT ↓59.4%), auto-induction (↑21% at week 2) **F1**: formulation (solution ↓45.6%) **BSV**: CL, V, MAT **BOV**: F1	332 samples, intensive (1st dose) + sparse (week 2+6)	33	Black, Nonblack
Hirt *et al.*[32] (2009)	POP-PK; NONMEM VI; 1-comp model with 1st order absorption and elimination	**CL**: ↑ weight (linear model), ↓ age, **F1**: formulation (solution ↓45.6%) **BSV**: CL, V	200 samples, sparse (all at day 15) + intensive (9 children at month 2 and 5)	48	African
Viljoen *et al.*[34] (2012)	POP-PK(/PGx); NONMEM VI; 1-comp model with 1st -order absorption and elimination	**CL**: ↑ weight[a], ↓ age, *CYP2B6 516G>T* (GT ↓37.2%, TT ↓66.4%), **V**: ↑ weight[a], ↓ age **BSV**: CL, V **BOV**: CL	649 samples, sparse, multiple OCC	60	African
Salem *et al.*[162] (2014)	POP-PK(/PGx); NONMEM VI; 1-comp model with 1st -order absorption and elimination	**CL**: ↑ weight[a], *CYP2B6 516TT* (↓51%),↑ age – maturation (90% of E_{max}[b] at 9 months) **F1**: formulation + age (liquid form 90% of $E_{max\text{-}sol}$[c] at 8 years + $E_{max\text{-}sol}$=0.79 $F1_{caps/tabs}$), formulation (solution ↓) **BSV**: CL, V, F1 **BOV**: CL	3172 samples, intensive (at weeks 2, 6, 10, 56 and 112) + sparse	96	Cauc., Black, Hisp., Native Amer.

[a]Allometric scaling, [b]Sigmoid E_{max} model, [c]E_{max} model

CL – clearance, V – volume of distribution, MAT – mean absorption time, F1 – bioavailability, OCC – PK sampling visit (relates to all samples taken within one dosing interval), Cauc. -Caucasian, Hisp. – Hispanic

Table 2.12 Review of population pharmacokinetic models for efavirenz in children (part 2)

Reference (year)	Model (type, software, PK)	Covariates	Data	n	Race
Luo *et al*.[291] (2016)	POP-PK(/PGx); NONMEM VI; 2-comp model with 1st -order absorption and elimination	**CL:** age group (↑children), ↑ weight[a], ↑ PART, *CYP2B6 516G>T* (GT↓ 24.4%, TT↓ 61.3%)† **V_{cent}:** age group (↑children), ↑ weight[a] **ka:** ↑weight[a] **F1: f**ormulation (solution ↓ 35-75% depending on study) **BSV:** CL, V_{cent}, Q, V_{per}, ka **RUV**: different variance terms depending on formulation and age group*	4521 samples (3289 in 168 children and 1232 in 24 adults) – intensive and sparse, multiple OCC	192	Cauc., Black, Other

[a]Power model, *age group = adults vs children, †despite significance not included in the final model

CL – clearance, PART – prior exposure to antiretroviral therapy in study PACTG1021, V_{cent} – volume of central compartment, ka – absorption rate constant, F1 – bioavailability, OCC – PK sampling visit (relates to all samples taken within one dosing interval), Cauc. -Caucasian, Q – intercompartmental clearance, V_{per} volume of peripheral compartment, RUV – residual unexplained variability

Table 2.13 Review of population pharmacokinetic models for efavirenz in adults (part 1)

Reference (year)	Model (type, software, PK)	Covariates	Data	n	Race
Villani *et al.*[163] (1999)	POP-PK; P-Pharm; 2-comp open model	None	115 samples, intensive (1 OCC)	22	NI
Barrett *et al.*[164] (2002)	POP-PK; NONMEM; 2-comp model with 1st order absorption and elimination	**CL**: ↑ at steady state, race (↓Asian +Black), sex (females ↓11%) **BSV**: CL, V	meta-analysis – 60% of 9342 samples	334	Asian, Black, Cauc., Other
Pfister *et at.*[172] (2003)	POP-PK/PD;NONMEM; 1-comp model with 1st-order absorption and elimination through well-stirred liver model (accounting for 1st pass metabolism)	**CL_{int}**: race (↓28% Black American and Hispanic) **F_{gut}**: adherence (↑4.3% for 100% adherence)	531 samples, intensive and sparse (multiple OCC)	139	Cauc, Black, Hisp, Asian
Csajka *et al.*[168] (2003)	POP-PK/PD(/PG); NONEMEM V; 1-comp model with 1st order absorption and elimination	No significant and clinically relevant covariates identified* **BSV**: F1	719 samples, intensive (7 individuals) + sparse (multiple OCC)	235	Cauc, Afr-Am, Hisp, Asian
Haas *et al.*[69] (2004)	POP-PK/PD(/PG); NMLE 3.2; 1-comp model with 1st order absorption and elimination	**CL**: *CYP2B6 516G>T* (GT ↓23%, TT ↓54%), *CYP3A5 6986 A>G* (AG ↑10%, GG ↑27%)	sparse at weeks: 1, 4, 12, 24	157	Cauc, Afr-Am, Hisp, Other
Haas *et al.*[185] (2005)	POP-PK/PD(/PG); NMLE 3.2; 1-comp model with 1st order absorption and elimination	None evaluated – explored the effect of covariates on model estimated AUC (affected by *CYP2B6 516G>T*)	sparse at weeks: 4 and 32	340	Cauc, Black, Hisp
Kappelhoff *et al.*[165] (2005)	POP-PK; NONEMEM V; 2-comp model with 3 transit comp for absortption, 1st order absorption into central comp and elimination	**F1**: race (↑56% for Asian, ↓10% for missing), baseline total bilirubin (↑57% if > 1.5-fold ULN) **BSV**: CL, V_{cent}, k_{tr}, Q **BOV**: F1	1009 samples, intensive (40 individuals – 694 samples) + sparse (315 samples)	172	Cauc, Black, Latino, Asian
Kappelhoff, van Leth *et al.*[66] (2005)	POP-PK; NONMEM, 1-comp model with 1st-order absorption and elimination	**CL**: steady state (↑10%), geographic location (Western countries ↑76%, South America + South Africa ↑53), nevirapine (↑43%) **BSV**: CL, V (both correlated)	1694 samples, intensive + sparse	376	South American, African, Asian, Cauc
Ribaudo *et al.*[376] (2006)	POP-PK(/PG); S-PLUS; 1-comp model with 1st -order absorption and elimination	**CL**: *CYP2B6 516G>T* ($t_{1/2\ GG}$=23h, $t_{1/2\ GT}$=27h, $t_{1/2\ TT}$=48h)	No information	152	Cauc, Afr-Am, Hisp

*significant effect of ABCB1 (MDR1-C3435T) on CL evaluated in 33 patients only

CL – clearance, V – volume of distribution, F1 – bioavailability, OCC – PK sampling visit (relates to all samples taken within one dosing interval), CL_{int} – clearance intrinsic, F_{gut} – pre-hepatic bioavailability, k_{tr} – transit rate constant, Q – inter-compartmental clearance, V_{cent} – central volume, ULN – upper limit of normal, Cauc. - Caucasian, Afr Am - African American, Hisp. – Hispanic, NI – not indicated

Table 2.14 Review of population pharmacokinetic models for efavirenz in adults (part 2 - continuation)

Reference (year)	Model (type, software, PK)	Covariates	Data	n	Race
Nyakutira *et al.*[36] (2008)	POP-PK(/PG); NONMEM VI; 1-comp model with 1st order absorption	**CL**: *CYP2B6 516G>T* (GT ↓23%, TT ↓57%) **BSV**: CL	64 samples sparse only (12 individuals 2 OCC)	58	African
Arab-Alameddine *et al.*[171] (2009)	POP-PK(/PG); NONMEM VI; 1-comp model with 1st order absorption and elimination	**CL**: *CYP2B6*, *CYP2A6*, *CYP3A4* rs4646437 (all SNPs included using square root models), weight (power 0.7) **BSV**: CL	393 samples all sparse (multiple OCC)	169	Cauc, Afr-Am, Hisp, Asian
Zhu *et al*.[166] (2009)	POP-PK; NONMEM; 2 comp with 1st order absorption and elimination using a well stirred liver model with auto-induction and interaction models	**CL**: auto-induction (CL↑ 60% at day 14, CL↑ 150% at day 35) and interaction with carbamzazepine at steady state (CL↑ 190%)	920 samples, intensive and sparse	37	No info
Cabrera *et al*.[377] (2009)	POP-PK(/PG); NONMEM VI; 1-comp model with 1st -order absorption and elimination	**CL**: *CYP2B6 516G>T* (GT ↓50%, TT ↓75%) BSV: CL, V	sparse only (2 – 7 per patient)	131	Cauc, Black
Mukonzo *et al.*[39] (2009)	POP-PK(/PG); NONMEM VI; 2-comp model with zero- followed by 1st -order absorption	**CL**: CYP2B6*6 (↓21%), CYP2B6*11 (↓20%) **F1**: *ABCB1* rs3842 (↑26%) **V_{per}**: sex (females ↑108%) **BSV**: CL, V_{cent}, V_{per}, Q, ka, F1, D1	402 samples – intensive (32 individuals), sparse (89 individuals)	121	African
Sánchez *et al.*[173] (2011)	POP-PK(/PG); NONMEM VI; 1-comp model with 1st -order absorption and elimination	**CL**: *CYP2B6 516G>T* (GT ↓39.8%, TT ↓64.6%), *MPR4 1497C>T* (↓20.7%), GGT (↓CL for ↑ GGT – cantered linear model) **BSV**: CL, V	869 samples	128	Cauc
Siccardi *et al.*[233] (2012)	POP-PK(/PG); NONMEM VI; 1-comp model with 1st -order absorption and elimination	**CL**: *CYP2B6 516G>T* (GT ↓39.8%, TT ↓64.6%) **BSV**: CL, V	202 - intensive (9 healthy volunteers) + sparse (148 patients)	157	No info

CL – clearance, V – volume of distribution, F1 – bioavailability, OCC – PK sampling visit (relates to all samples taken within one dosing interval), Q – inter-compartmental clearance, GGT - gamma-glutamyltranspeptidase, V_{per} – peripheral volume, V_{cent} – central volume, ka – absorption rate constant, D1 – duration of zero-order absorption phase, Cauc. -Caucasian, Afr Am - African American, Hisp. – Hispanic, NI – not indicated

Table 2.15 Review of population pharmacokinetic models for efavirenz in adults (part 3 - continuation)

Reference	Model (type, software, PK)	Covariates	Data	n	Race
Bertrand *et al.*[170] (2013)	POP-PK(/PG); NONMEM VI; 1-comp model with zero-order delayed absorption and 1st -order elimination	**CL**: *CYP2B6 516G>T* – effect modified by TB treatment and NAT2 metaboliser type, CYP2B6 485-18T (↓ 30%), weight (linear centred) **BSV**: CL **BOV**: CL	1111 samples - intensive (10 individuals at week 50) and sparse (week 2, 6, 22 and 50)	307	Cambodian
Mukonzo *et al.*[194] (2014)	POP-PK(/PG); NONMEM VI; 1-comp model with 1st -order absorption and elimination	**CL**: CYP2B6*6/*1 (↓21%), CYP2B6*6/*6 (↓54%) **F1**: *ABCB1* rs3842 (↑22%) **BSV**: CL	556 samples sparse	99	African
Dooley *et al.*[71] (2014)	POP-PK(/PG); NONMEM VII; 1-comp model with 1st -order absorption and elimination through well stirred liver model	**CL_{int}**: composite *CYP2B6 516GT|983TC*[b] (IM ↓41%, SM ↓74%, USM ↓914%), pregnancy (↑19%), isoniazid and slow NAT2 metaboliser type (↓21%), weight[a] **V**: weight[a] **BSV**: CL **BOV**: F_{gut}	468 samples - sparse (up to 4 per sampling OCC)	87	African (pregnant women)
Dickinson *et al*.[103] (2015)	POP-PK(/PG); NONMEM VII; 1-comp model with 1st -order absorption and elimination	**CL**: metaboliser status based on composite *CYP2B6 516GT|983TC* and *CYP2A6*9B/*1*, weight[a] V: weight[a] **BSV**: CL **BOV**: CL	1491 samples - sparse + intensive (46 individuals)	606	African, Asian, Cauc, Hisp
Hui *et al*.[223] (2016)	POP-PK(/PG); NONMEM VII; 1-comp model with 1st -order absorption and elimination	**CL**: *CYP2B6 516G>T* (GT ↓29%, TT ↓77%), weight **BSV**: CL, V	266 samples sparse + intensive (9 individuals)	163	Chinese

[a]Allometric scaling, [b]composite 516GT|983TC metaboliser classification: EM (extensive metabolisers) - 516GG|983TT; IM (intermediate metabolisers) - 516GG|983TC or 516GT|983TT, SM (slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (ultra-slow metabolisers) - 516GG|983CC.

CL – clearance, V – volume of distribution, F1 – bioavailability, F_{gut} – pre-hepatic bioavailability, OCC – PK sampling visit (relates to all samples taken within one dosing interval), CL_{int} – clearance intrinsic, NAT2 – N-acetyl transferase type 2, Cauc. -Caucasian, Afr Am - African American, Hisp. – Hispanic, NI – not indicated

Table 2.16 Review of population pharmacokinetic models for nevirapine in children

Reference (year)	Model (type, software, PK)	Covariates	Data	n	Race
Chokephaibulkit *et al*.[366] (2005)	POP-PK; NONMEM ; 1-comp model with 1st-order absorption and elimination	No information	No information	34	Thai
Foissac *et al.*[374] (2012)	POP-PK; MONOLIX 4.0; 1-comp model with 1st-order absorption and elimination	**CL**: ↑ weight[a] **V**: ↑ weight[a] **F1**: ↑ age[b] (90% maturation at 4 years) **BSV**: CL	360 samples sparse and intensive	94	NI
Nikanjam *et al.*[305] (2012)	POP-PK(/PG); NONMEM VI; 1-comp model with 1st-order absorption and elimination	**CL**: ↑ weight[a], ↑ age[b] (39% at birth, 100% maturation at 4 years), *CYP2B6 516G>T* (516TT ↓36%), ritonavir (↓ 25%) **F1**: Triomune formulation (↓ 42%) **V**: ↑ weight[a] **BSV**: CL, V (correlated) **BOV**: CL	3759 samples (sparse and intensive)	639	African, Asian, Cauc, Hisp

[a]Allometric scaling, [b]Sigmoid E_{max} model

CL – clearance, V – volume of distribution, F1 – bioavailability, OCC – PK sampling visit (relates to all samples taken within one dosing interval), Cauc. -Caucasian, Hisp. – Hispanic, NI – not indicated

Table 2.17 Review of population pharmacokinetic models for nevirapine in adults (part 1)

Reference (year)	Model (type, software, PK)	Covariates	Data	n	Race
Zhou *et al.*[295] (1999)	POP-PK; NONMEM V, 1-comp model with zero-order absorption and 1st-order elimination	**CL**: sex (male ↑1.31) **BSV**: CL	273 samples (sparse but measured at different time at each visit, multiple OCC)	82	Cauc, Black, Hisp
de Maat *et al.*[296] (2002)	POP-PK; NONMEM V, 1-comp model with 1st-order absorption and elimination	**CL**: HEP-C (↓52%), ASAT>1.5x ULN (↓34%), race (black ↓27%), ↑ weight (0.21 L/h for 10kg) **BSV**: CL, kel **BOV**: CL, V	1329 samples (intensive – 13 patients + 757 sparse samples, multiple OCC)	173	Cauc, Black, Asian
Kappelhoff, van Leth *et al.*[66] (2005)	POP-PK; NONMEM, 1-comp model with 1st-order absorption and elimination	**CL**: steady state (↑39%), geographic location (Western countries ↑28%, South America ↑11), sex (females ↓13.8%), HEP-B (↓ 19.5%) **BSV**: CL, V (correlated)	3024 samples (intensive and sparse, multiple OCC)	701	South American, African, Asian, Cauc
Capparelli *et al.*[378] (2008)	POP-PK; NONMEM V, 1-comp model with 1st-order absorption and elimination	No covariates **BSV**: CL, ka	intensive sampling	26	Cauc, Black, Hisp
Moltó *et al.*[297] (2008)	POP-PK; NONMEM V, 1-comp model with 1st-order absorption and elimination	**CL**: ↑ weight (linear) **V**: ↑ weight (linear) **BSV**: CL, V, ka	319 (intensive, 1 OCC)	40	Cauc
Elsherbiny *et al.*[50] (2009)	POP-PK; NONMEM VI, 1-comp model with 1st-order absorption and elimination	**CL**: rifampicin (↑37.4%), circadian variation (night ↑27%), ↑ age (1.56% per year), ↑ albumin (2.84% per 1 g/L), ↑ vit B (11.8%) **ka**: rifampicin (↓6x), circadian variation (night ↓10x) **LAG**: circadian variation (day – no LAG, night - 0.73h) **V**: ↑ weight (1.42% per 1 kg) **BSV**: CL, ka	(sparse and intensive in 8 patients)	53	African

CL – clearance, V – volume of distribution, F1 – bioavailability, HEP-C – hepatitis C, HEP-B – hepatitis B, ULN – upper limit of normal, ASAT - aspartate transaminase, kel – elimination rate constant, ka – absorption rate constant, OCC – PK sampling visit (relates to all samples taken within one dosing interval), LAG – delay in absorption, Cauc. -Caucasian, Hisp. – Hispanic, NI – not indicated

Table 2.18 Review of population pharmacokinetic models for nevirapine in adults (part 2 - continuation)

Reference (year)	Model (type, software, PK)	Covariates	Data	n	Race
Chou *et al.*[298] (2010)	POP-PK(/PG); MONOLIX 2.4; 1-comp model with 1st-order absorption and elimination	**CL**: *CYP2B6 516G>T* (516GT ↓11%, 516TT ↓37%), ↑ creatinine clearance (power model) **BSV**: CL **BOV**: CL	sparse and intensive data (10 patients), 2 OCC	170	Cambodian
Schipani *et al.*[73] (2011)	POP-PK(/PG); NONMEM VI; 1-comp model with 1st-order absorption and elimination	**CL**: *CYP2B6 516G>T* (516GT ↓14%, 516TT ↓37%, 983TC↓40%), weight (5%↓ for 10kg) **BSV**: CL	403 samples (sparse + 9 patients intensive, 1 OCC + 11 patients 2 OCC)	270	Cauc, Black
Lehr *et al.*[300] (2011)	POP-PK(/PG); NONMEM VI; 1-comp model with 1st-order absorption and elimination	**CL**: *CYP2B6 516G>T* (516GT↓19.4%, 516TT↓30.6%), CYP2C19 (HOM ↓26.8%), race (Black/Asian ↓19.4%), **BSV**: CL, V (correlated)	1260 samples (sparse and intensive)	271	South Amer, African, Asian, Cauc
Svennson *et al.*[294] (2012)	POP-PK(/PG); NONMEM VII; 1-comp model with absorption through 2 transit comp and elimination	**CL**: weight (fat free mass)[a], mixture model (slow + extensive metabolisers) **F1**: TB treatment (↓ 39%) **V**: weight[a] **MTT**: food (2.5h fed, 0.6h fasted) **BSV**: CL, F1 **BOV**: F1, MTT	1270 samples (sparse and intensive)	115	African
Dickson *et al.*[72] (2014)	POP-PK; NONMEM V, 1-comp model with 1st-order absorption and elimination	**CL**: *CYP2B6 516G>T|983T>C* (516TT|983TT ↓23%, 516GT/GG|983TC ↓36%) **BOV**: CL **BSV**: CL	sparse and intensive – 40 patients, multiple OCC	180	Malawi

[a]Allometric scaling

CL – clearance, V – volume of distribution, F1 – bioavailability, HOM – homozygote, MTT – mean transit time, TB – tuberculosis, OCC – PK sampling visit (relates to all samples taken within one dosing interval), LAG – delay in absorption, Cauc. -Caucasian, Hisp. – Hispanic, NI – not indicated

CHAPTER 3: METHODS

This thesis is supported by four peer reviewed publications (Chapter 4 - 7), the content of each one is included in its unchanged version with the abstract, introduction, methods, results and discussion sections in full. The methods sections in each of the publications present following information:

Chapter 4 (**Publication/study 1**) presents information relevant to the data on efavirenz pharmacokinetics in studies CHAPAS-3 and ARROW. This includes: efavirenz dosage, formulations, PK sampling schedule, transport and storage of samples, details of assays used for quantification of efavirenz plasma concentrations, details of procedure and assays used for genotyping, details of population pharmacokinetic modelling used for data analysis (structural model building and diagnostic, covariate analysis and simulations).

Chapter 5 (**Publication/study 2**) presents information relevant to the data on outcome of treatment with efavirenz in CHAPAS-3. This includes: study overview and viral load sampling schedule, details of statistical analysis (inclusion criteria for data, statistical tests used for between group comparisons, details of Cox proportional hazards regression modelling used for detection of associations with main outcome [VL > 100 copies/mL], details of likelihood profiling method developed for identification of drug exposure most predictive of increased risk of non-suppression, details of multivariate analysis).

Chapter 6 (**Publication/study 3**) presents information relevant to the data on nevirapine pharmacokinetics in studies CHAPAS-3 and CHAPAS-1. This includes: nevirapine dosage, formulations, PK sampling schedule, transport and storage of samples, details of assays used for quantification of nevirapine plasma concentrations, details of procedure and assays used for genotyping, details of population pharmacokinetic modelling used for data analysis (structural model building and diagnostic, covariate analysis and simulations).

Chapter 7 (**Publication/study 4**) presents information relevant to the data on outcome of treatment with nevirapine in CHAPAS-3. This includes: study overview and viral load sampling schedule, details of statistical analysis (inclusion criteria for data, statistical tests used for between group comparisons, details of Cox proportional hazards regression modelling used for detection of associations with main outcomes [VL > 100 copies/mL and >1 transaminase elevations], modifications to procedure for identification of drug exposure most predictive of increased risk of developing main outcomes, details of multivariate analysis).

Non-linear mixed effects modelling was conducted with the software NONMEM VII (version 7.3), and facilitated using Perl Speaks NONMEM (PSN) 4.4.0.[379] Data exploration, plotting, processing of model run results, statistical tests and Cox proportional hazards regression modelling were conducted with

R.[380] Additionally, fractional polynomials in STATA 13 or 14 (StataCorp. 2013/2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP) were used to explored non-linearities in effects of continuous covariates in Cox modelling.

Chapter 3 presents further details of the clinical studies included in this thesis (study design, objectives, population and inclusion/exclusion criteria, chronology of the studies) and elaborates on the statistical methods employed with justification of their choice.

3.1 Clinical Studies[l]

The main clinical study supporting this thesis is CHAPAS-3 (data previously not analysed in relation to nevirapine and efavirenz). In order to increase statistical power in the population pharmacokinetic analysis the data from CHAPAS-3 was enriched with the data from pharmacokinetic sub-studies of CHAPAS-1 (to describe nevirapine PK) and ARROW (to describe efavirenz PK). The studies are presented in chronological order.

3.1.1 CHAPAS-1

CHAPAS-1 (Children with HIV in Africa – Pharmacokinetics and Adherence of Simple Antiretroviral Regimens) was an open-label, controlled phase I/II trial randomising 212 HIV-infected African children to start nevirapine treatment with either dose escalation schedule of once-daily administration for 14 days (followed by full dose twice a day) or full dose twice a day without escalation (randomisation ratio 1:1).[33] All children were treated with new paediatric FDC tablets (Triomune Baby/Junior). The CHAPAS-1 trial diagram is presented in Figure 3.1

The overall aim of the CHAPAS-1 was to evaluate the appropriate dosing of, and adherence to, the new formulation combining stavudine (d4T), lamivudine (3TC), and nevirapine (NVP) in a dispersible FDC tablet specifically developed for children (Triomune Baby/Junior). The specific objectives were to describe the toxicity related to nevirapine, in order to investigate the necessity of dose escalation in African children treated with new FDC tablets and to determine the pharmacokinetics of the new formulation, as well as to describe possible interactions with common concomitant medications, such as rifampicin. Further objectives were to describe mortality, disease progression, hospital admission rates and laboratory markers after initiation of ART and to estimate the budget impact and cost-effectiveness of effective ART in HIV infected children in Zambia.

The study was conducted at a single site in Zambia (University Teaching Hospital, Lusaka) between February 2006 and October 2008. All children were treated with the new paediatric FDC tablets Triomune Baby/Junior and dosed according to WHO 2006 recommendations.[56]

The study enrolled children aged 3 months to 14 years inclusive (<30 kg in weight), previously untreated with ARVs and fulfilling one of the WHO 2006 criteria for treatment initiation (related to HIV disease stage and CD4%).[56] Prior exposure to nevirapine for prevention of mother to child transmission (pMTCT) was an exclusion criterion. After enrolment, children were followed up for at

[l] Studies presented in chronological order.

least 48 weeks. 64 children (16 per age group, i.e. <3 years, 3-6 years, 7-10 years, 11-14 years) were also enrolled in a 12 hour PK sub-study with 7 blood draws at least 4 weeks after starting ART.[77] An additional 14 children, weighing 3-6 kg were enrolled into a separate 12 hour PK sub-study involving only 4 blood draws on account of their size.[37] The objective of the intensive PK sub-study was to describe nevirapine exposures in the dosing weight bands suggested in WHO 2006 guidelines.[56]

The data used in this thesis was limited to the PK sub-study of CHAPAS-1, the details of the sampling schedule are presented in Chapter 6.

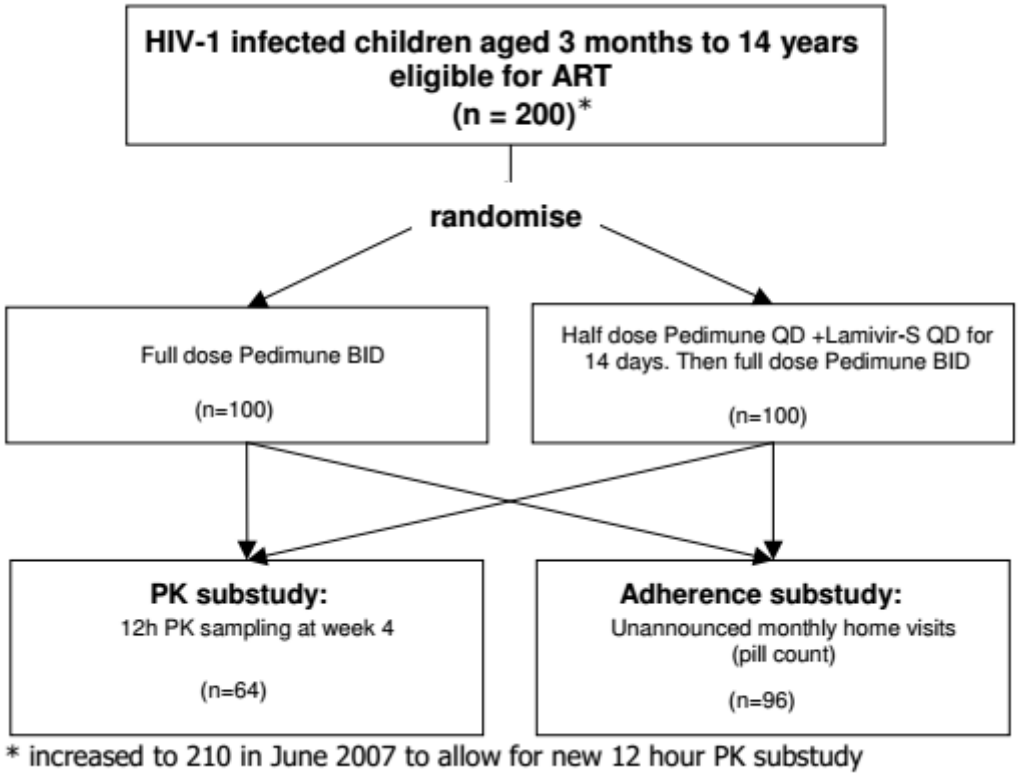

Figure 3.1 CHAPAS-1 trial diagram (reproduced from the CHAPAS-1 trial protocol with permission authors' permission). Pedimune = Triomune Baby/Junior (lamivudine + stavudine + nevirapine), Lamivir-S = (lamivudine + stavudine).

3.1.2 ARROW

ARROW (Antiretroviral Research for Watoto[J]) was an open-label, parallel group, multicentre randomised controlled clinical trial in 1200 symptomatic HIV infected African infants.[381] The trial had a complicated design with a number of randomisation stages and its main objective was primarily to evaluate two strategic approaches for management of ART in children: the first strategy compared clinically driven monitoring (CDM) with laboratory plus clinical monitoring (LCM); the second approach

[J] Watoto means "Children" in Swahili language.

compared a continuous first line ART three drug/two class regimen (2 nucleoside reverse transcriptase inhibitor [NRTIs] +1 non-nucleoside reverse transcriptase inhibitor [NNRTI]) with a four drugs/two classes induction (3 NRTIs +1 NNRTI) followed by maintenance with three drugs. Additionally, after at least 36 and 96 weeks on ART respectively, two further randomisations assessed simplification strategies hypothesised to improve long-term ART adherence: (i) once versus twice daily abacavir (ABC) + 3TC, (ii) stopping versus continuing daily co-trimoxazole prophylaxis. The ARROW trial diagram visualising primary (first stage) and secondary (second stage) randomisations are presented in Figures 3.2 and 3.3 (respectively).

The study was run at four sites in Africa: 3 in Uganda (Medical Research Council/Uganda Virus Research Institute, Entebbe; Joint Clinical Research Centre, Kampala; Paediatric Infectious Disease Clinic/Mulago, Kampala) and 1 in Zimbabwe (University of Zimbabwe-Clinical Research Centre, Harare) between March 2007 and March 2012. All children were dosed according to WHO 2006 recommendations[56] and where available they were treated with paediatric FDC tablets (3-in-1 or 2-in-1).

The study enrolled children aged 3 months to 17 years inclusive (13-17 years were capped at 10%), previously untreated with ARVs and fulfilling one of the WHO 2006 criteria for treatment initiation (related to HIV disease stage and CD4%),[56] with or without prior exposure to nevirapine for pMTCT.[61] After enrolment children were followed up for 3½-5 years. 41 children from the main study (aged 3–12 years) treated with a combination of EFV+3TC+ABC took part in the intensive PK sub-study. The objective of the sub-study was to describe abacavir exposures in the dosing weight bands suggested in WHO 2006 guidelines[56] under once a day and twice a day dosing and included children limited to 2 Ugandan sites (Kampala and Mulango). Children were sampled for abacavir on two separate occasions (at week 36 and 4 weeks later). The companion drugs to abacavir used in individual children were additionally measured in the same blood samples, this included efavirenz concentrations.

The data used in this thesis was limited to the efavirenz concentrations from the PK sub-study of ARROW, the details of the sampling schedule are presented in Chapter 4.

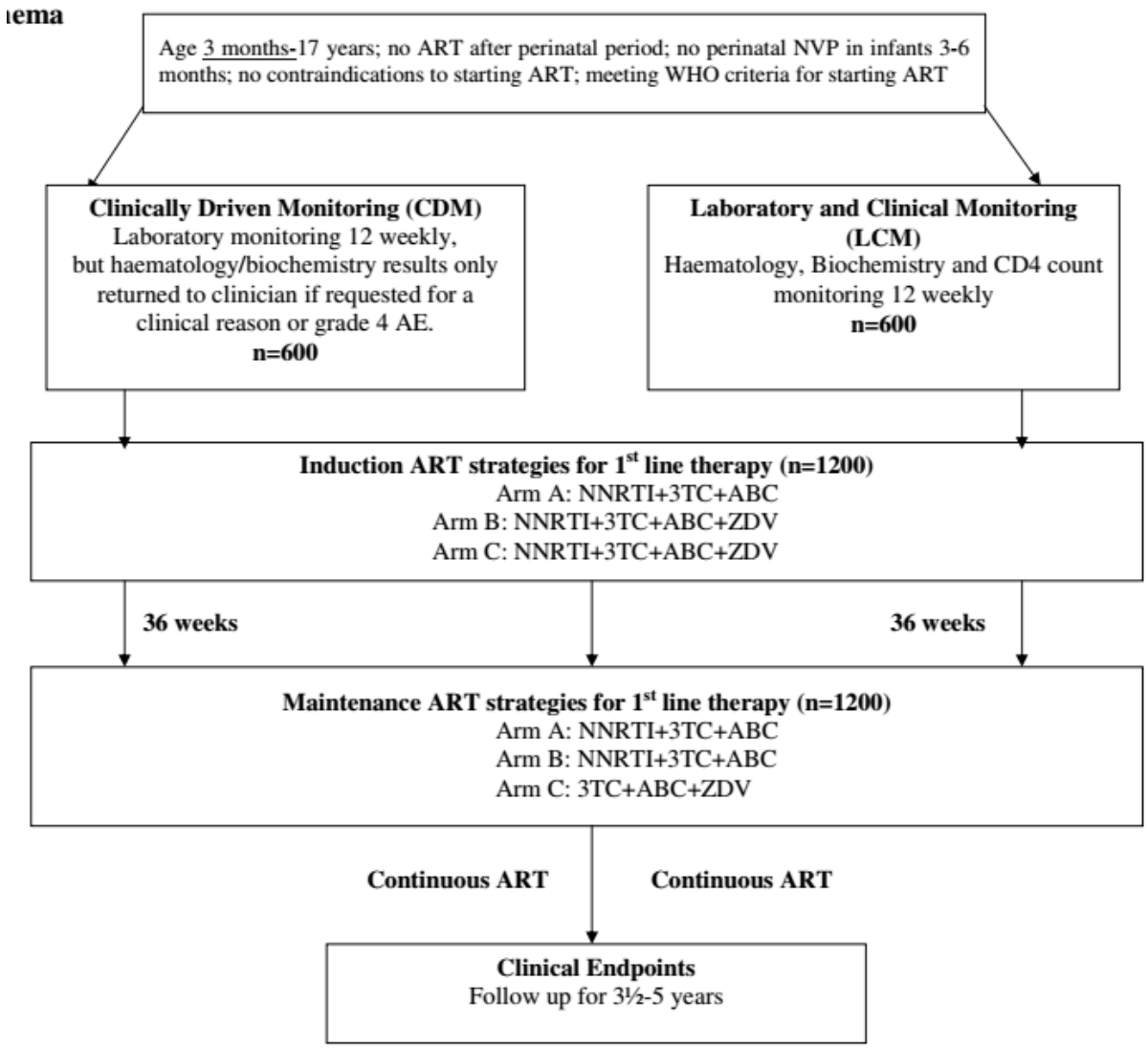

Figure 3.2 ARROW trial diagram part 1 depicting the primay randomisation (reproduced from the ARROW trial protocol with permission authors' permission). ART- antiretroviral treatment, AE – adverse event, NRRTI – non-nucleoside reverse transcriptase inhibitor, 3TC - lamivudine, ABC – abacavir, ZDV - zidovudine.

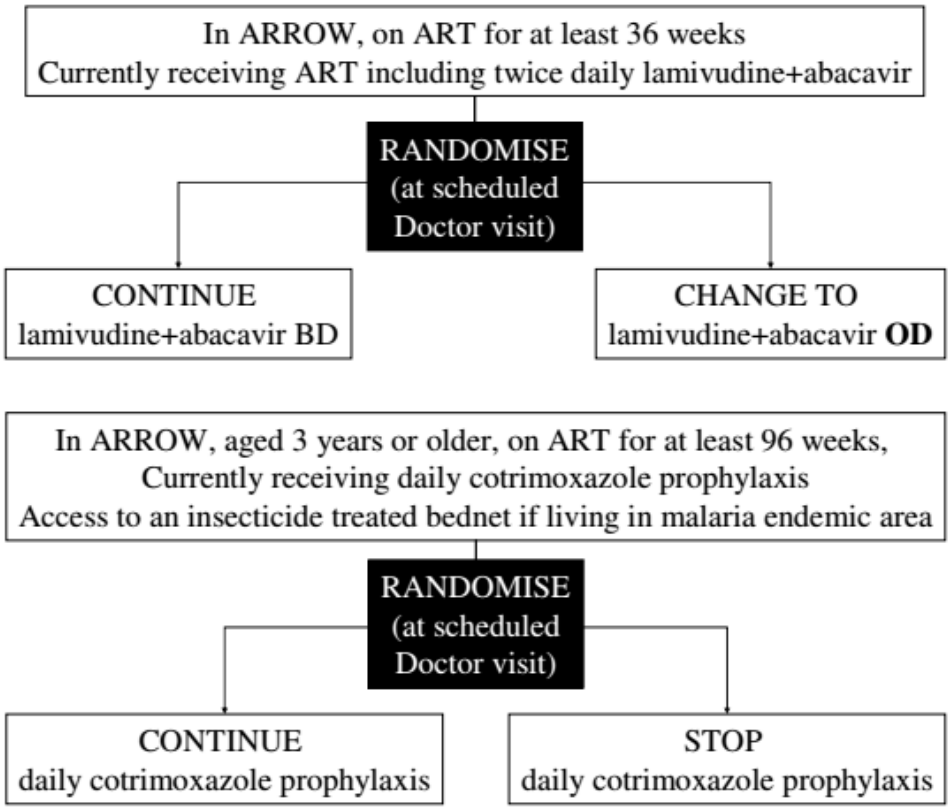

Figure 3.3 ARROW trial diagram part 2 depicting the secondary randomisation (reproduced from the ARROW trial protocol with permission authors' permission). BD – twice daily, OD – once daily, ART – antiretroviral treatment

3.1.3 CHAPAS-3

CHAPAS-3 (Children with HIV in Africa – Pharmacokinetics and Adherence of Simple Antiretroviral Regimens) was an open-label, multicentre, randomised, controlled phase II/III trial in 478 African infants and children.[382] The main objectives of the study were to compare the pharmacokinetics, toxicity, acceptability, adherence, virological efficacy and cost-effectiveness of the three first-line two drug ART (NNRTI + 2 NRTIs) - combinations of NVP or efavirenz (EFV) with 3TC and ABC, or d4T, or zidovudine (ZDV). The aim of the trial was to provide the evidence for the FDA and WHO review of these new formulations and endorse the WHO recommendations for simplified dosing.[11] In addition the study aimed to describe the pharmacokinetics of efavirenz in the new paediatric dispersible double scored 600 mg tablets (allowing tablets to be split to 400 mg, 300 mg or 200 mg doses). The trial diagram is presented in Figurfe 3.4.

The study was run at four sites in Africa: 3 in Uganda (Joint Clinical Research Centre, Kampala; Bristol Myers Squibb Children's Clinical Centre of Excellence, Baylor College of Medicine, Kampala; Joint Clinical Research Centre, Gulu) and 1 in Zambia (University Teaching Hospital, Lusaka) between November 2010 and December 2013. All children were dosed according to WHO 2010 recommendations[56] exclusively with paediatric dispersible (3-in-1 or 2-in-1) FDC tablets and/or double scored paediatric efavirenz tablets.

The study enrolled children aged 3 months to 13 years inclusive (3 – 13 years for efavirenz), either previously untreated (ART-naïve) or treated with d4T-based regimens (NNRTI + 2 NRTIs) for at least 2 years and virologically suppressed (viral load < 50 copies/mL) at screening. The protocol allowed inclusion of children with or without prior exposure to nevirapine for pMTCT (children aged 3 -12 months included only if not exposed to pMTCT). ART-naïve children had to meet one of the WHO 2010 criteria for treatment initiation (related to HIV disease stage and CD4%).[56] After enrolment all children were followed up for at least 2 years. All children in the study took part in sparse PK sampling (2 PK samples taken about 2h apart at each visit at weeks 6, 36, and every 24 weeks thereafter until the end of follow up). Additional intensive PK-sampling was conducted in the first patients enrolled in each WHO weight-band at week 6 and in all patients who acquired tuberculosis during the study (4-10 weeks after tuberculosis treatment initiation and again 4-10 weeks after tuberculosis treatment cessation). Viral loads were measured at baseline, week 48, week 96 and week 132 (or 144).

The details of the formulations, dosage, sampling schedules and essays can be found in Chapters 4 – 7.

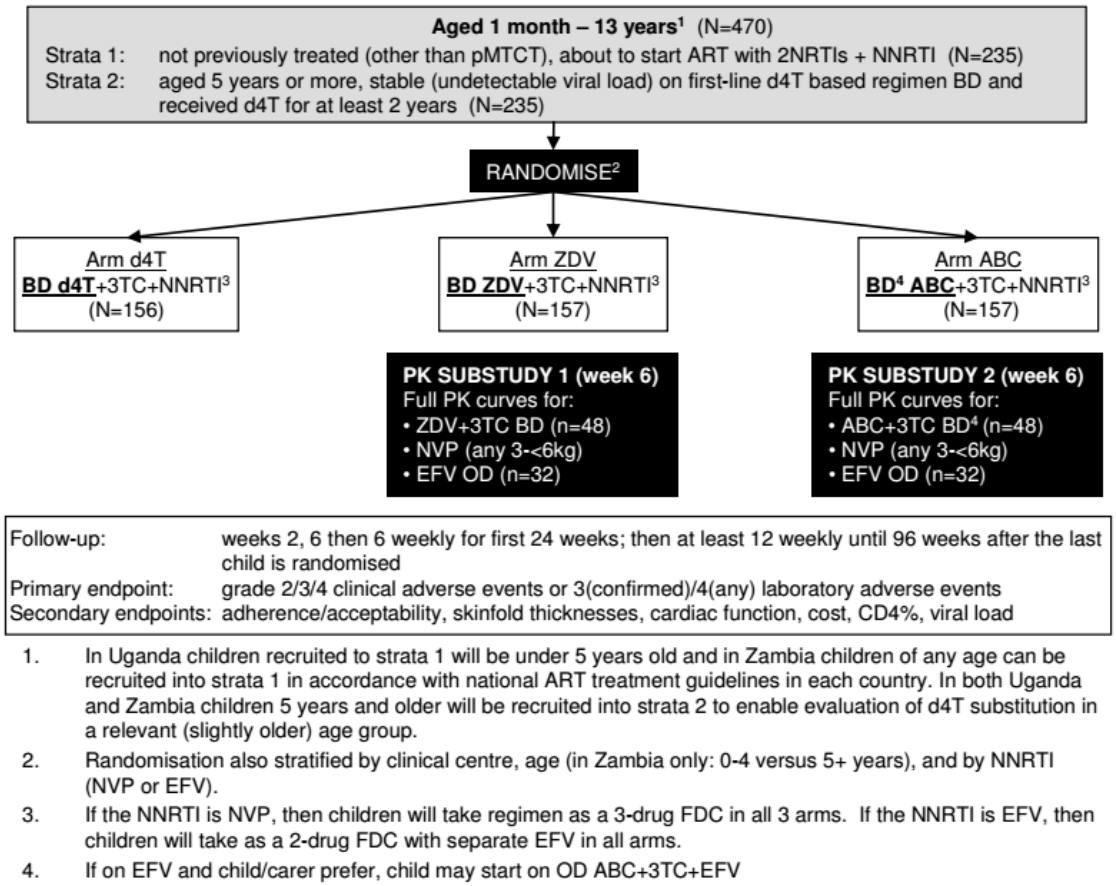

Figure 3.4 CHAPAS-3 trial diagram (reproduced from the CHAPAS-3 trial protocol with permission authors' permission). d4T - stavudine, BD – twice a day, 3TC - lamivudine, NNRTI – non-nucleoside reverse transcriptase inhibitor, ZDV - zidovudine, ABC - abacavir, NVP - nevirapine, EFV - efavirenz, OD –once a day.

3.2 Data Analysis – choice of methods

3.2.1 Population pharmacokinetic modelling

Drug testing in children cannot be conducted in the same way as in adults. There are several practical and ethical constraints, for example the number of venipunctures per patient or amount of blood that can be drawn from a child. In order to overcome them, International Conference on Harmonisation (ICH) urged development and implementation of novel approaches to drug testing.[19,383] Population pharmacokinetic/pharmacodynamic (POP-PK/PD) analysis is one of them. POP-PK/PD analysis is a model-based approach allowing the description of the relationship between drug dose, concentration, and response a mathematical language. The model-based approach assumes that the human body is a structural entity creating a system of pathways linked together, which can be expressed through mathematical functions. In this approach, data from all individual patients is combined and analysed simultaneously to obtain PK or/and PD parameter estimates characterising the whole population.

POP-PK/PD analysis is also known as nonlinear mixed-effects modelling. Nonlinear refers to the fact that the relationship between the model parameters and the response is generally nonlinear[384], while mixed-effects refers to the fact that the model is constituted of both fixed and random effects. This technique enables not only to calculate values of population and individual pharmacokinetic parameters but also to identify and quantify various sources of variability in drug response: between subject-variability (BSV), between-occasion variability (BOV) and residual unexplained variability (RUV).[384–386] Additionally, it allows to characterise factors (covariates) explaining those differences, detect what PK parameters they influence, and quantify their effect. This facilitates to estimate the typical concentration-time profiles for the whole population, sub-groups within the population and in the individual patients. It is also a more mechanistic approach based on compartmental distribution of the drug in the body. In addition, this method is superior to classical regression analysis by allowing for the aforementioned non-linearity in associations between variables.[384]

A POP-PK model comprises of three building blocks. The first one is a "structural model", which contains basic equations describing the "mechanism" underpinning the PK and/or PD of the drug. The second one is a statistical component that models the variability in drug response around population values, which is explained by BSV/BOV and measurement error. The third one is a covariate sub-model, which incorporates influence of various factors, such as demographic, patho-physiological or genetic characteristics on the structural model. As the name "mixed effects" implies there are two kinds of effects incorporated in the model: fixed effects and random effects. Fixed effects are deterministic parameters that characterise the whole population, while random effects are stochastic in nature, and describe the differences between patients, occasions, or the measurement error affecting the observed data. Fixed effects are generally denoted with THETA (Θ) (*Equations 3.2* and *3.4*). Those are population parameter values, e.g. the typical values of clearance or volume of distribution, as well as the covariate effects explaining the variability in drug response - known, observed properties that vary across population (e.g. weight, *Equation 3.4*). Random effects are the variables that describe the aforementioned different levels of variability (BSV and BOV), and are generally denoted ETAs (*Equations 3.5* and *3.6*, respectively) or EPSILON for RUV (*Equation 3.2*). Combination of all those parameters enables to describe the population trends as well as individual patient response (Figure 3.5).

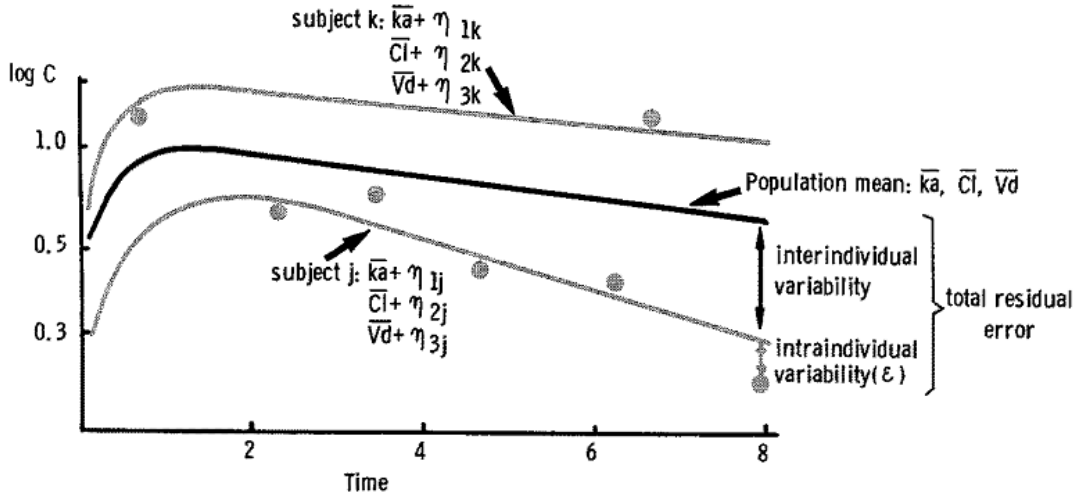

Figure 3.5 Graphical illustration of the statistical model used in NONMEM for the special case of a one-compartment model with first order absorption. Black: serum concentration curve resulting from the average population parameters; grey: serum concention curves of 2 subjects with different pharmacokinetic parameters (from Vozeh et al.[387])[K]

Note: THETAS here presented as: $\overline{\boldsymbol{ka}}$, population value of absorption rate constant; $\overline{\boldsymbol{Cl}}$, population value of clearance: $\overline{\boldsymbol{Vd}}$, population value of volume of distribution. Figure 3.5 presents only 2 levels of variability: η, inter-individual variability, ε – intra-individual variability (which in fact depicts residual unexplained variability). The true residual error should include three levels of variability: BOV, BSV and RUV.

The structural (parameter) model, comprising the first building block, can be simplified though following mathematical function:

$$y = f(\Theta, x)$$

Equation 3.1

where y is the observation, which is a function of parameter values (Θ) and known quantities x (being time, dose, dosing schedule, etc).

[K] Reprinted from the *Eur J Clin Pharmacol.,* 23, 445–451, Vozeh, S. *et al*., Population pharmacokinetic parameters in patients treated with oral mexiletine. Page No. 447, Copyright (1982) with permission from Springer Verlag.

As outlined above nonlinear mixed-effects modelling accounts for different level of variability and as a consequence the basic model is expanded as follows:

$$y = f(\Theta, x) + g(\phi, z, \Theta, x) + \varepsilon$$

Equation 3.2

where $f(.)$ is the structural model function, $g(.)$ is the variance function that relates the vector of known quantities x, the covariates z, the structural model parameters Θ, and residual variance model parameters ϕ to the variance of y, whereas ε indicates the residual error model.[388]

Equation 3.2 can be broken down to particular sub-models describing relations for individual predictions, covariate effect and variability. Model for estimating individual PK parameters can be summarised as:

$$y_j = f\left(\phi_j, x_j\right) \bullet \left(1 + \varepsilon_j(1)\right) + \varepsilon_j(2)$$

Equation 3.3

where y_j is the observed dependent variable (observation) in the *j*th individual when the PK parameters take the value ϕ_j (i.e. individual parameter prediction) under the vector of knows relations x_j. The RUV ($\varepsilon, \mathrm{EPSILON}$) is assumed to be normally distributed with a mean of zero and a variance of σ^2 (SIGMA), ε=(N, σ^2).[389] In *Equation 3.3* RUV has a combined error structure comprising of proportional error ($\varepsilon_j(1)$) and additive error ($\varepsilon_j(2)$).[388]

Each PK parameter can be described using a general model for fixed effects to account for covariate effect:

$$\phi_j = h\left(\Theta, z_j\right)$$

Equation 3.4

where ϕ_j is a PK parameter for *j*th individual patient, which is a function of the true (but unknown) population mean value (Θ) and a specific covariate (z_j), which could be either categorical (e.g. sex) or continuous (e.g. age) – the covariate model. *Equation 3.4* represents the base parameter model.

The model in *Equation 3.3* can then be expanded in terms of the aforementioned BSV, and so deviation of parameters ϕ_j for a particular individual from the population value can be expressed as follows:

$$\phi_j = h(\Theta, z_j) \bullet exp^{\eta_j}$$

Equation 3.5

where η_j is the BSV for the j^{th} individual and is assumed to be normally distributed with a mean of zero and a variance of $\boldsymbol{\omega^2}$ (OMEGA), η=(N, $\boldsymbol{\omega^2}$). BSV is usually modelled with an exponential function (what prevents implausible negative values of PK parameters), but depending on the character of the variability it can be modelled using another transformation approximating normal distribution (e.g. log-normal or logit). Equation 3.5 is the mathematical function describing the graphical illustration of model presented on Figure 3.1, where the individual pharmacokinetic parameters (ϕ_j) are $\overline{ka}$, $\overline{Cl}$ and $\overline{Vd}$, and BSV is η_j.

In addition model parameters can change between sampling occasions, which be described in terms of BOV,[390] and so *Equation 3.5* can be further expanded as follows:

$$\phi_{jk} = h(\Theta, z_{jk}) \bullet exp^{\eta_j + \kappa_{jk}}$$

Equation 3.6

where κ_{jk} describes the inter-occasion variability for j^{th} individual and k^{th} occasion, which is similarly assumed to be normally distributed with a mean of zero and a variance of π^2, κ=N(0, π^2). The temporal differences between *Equation 3.5* and *Equation 3.6* are indicated by the *k* subscript and can be quantified in longitudinal data with repeated sampling visits (relating to different dosing intervals).

An additional advantage of POP-PK modelling over classical analysis methods is that it enables analysis of sparse and unbalanced data [391] - by use of POP-PK models we can obtain full drug concentration curves by collecting as little as two samples per patient.[392] In addition, it enables combining data from various sources, such as: *in vitro*, *in vivo*, literature information, different studies or populations.[393] In contrast using a classical "two stage" analysis would not allow to combine pharmacokinetic data from various studies independent of their sampling schedules.[384] In a "two stage" method each individual's data is analysed individually and then averaged for the population and it requires identidal samplinch schedules for all analysed individuals. Another advantage is that it allows for graphical display of the

data and results, which makes decision-making easier and information understandable also to clinical audience with limited understanding of mathematics or statistics.[393]

Validated models can also be used for simulations of drug response under different dosing conditions or in other sub-populations and to allow testing of various "what-if" scenarios not feasible or ethical to test in real life.[384,394,395] Such simulations can facilitate treatment optimisation[18] and can be used to optimise trial design, minimising the numbers of individuals needed in a study.[385,396,397]

The outlined characteristics are particularly advantageous in drug testing in children, as they result in increased safety and feasibility of studies and reduction of burden related to drug testing.[384,393,398]

The most popular software used to conduct non-linear mixed effects modelling (also in the presented thesis) is NONMEM (Beal, S., Sheiner, L.B., Boeckmann, A., & Bauer, R.J., NONMEM User's Guides. [1989-2009], Icon Development Solutions, Ellicott City, MD, USA, 2009.) The presented nomenclature (THETA, ETA, SIGMA, OMEGA etc.) is utilized by users of this software and other programmes might use a different nomenclature.

3.2.2 Cox proportional hazards regression model

The most basic analysis method describing a relationship between one (or more) explanatory variable(s) and a response variable is linear regression for continuous outcomes, or logistic regression for binary outcomes (describing an association in a probabilistic manner). Simple regression analysis is based on a number of assumptions, one of them being that the relationship between a predictor and outcome is constant over the range of predictor variable, another is independence of data (each observation in the model should come from a different individual). Analysis of longitudinal data including repeated measurements and non-linear effects requires a more advanced approach.

One such approach is Cox proportional hazards regression model,[399] where probability of an effect is estimated in terms of hazard expressed through following function:

$$h(t, X) = h_0(t)\exp\left(\sum_{i=1}^{p} \beta_i X_i\right)$$

Equation 3.7

Where the hazard is a function of baseline hazard h_0 at a time t and sum of individual effects β_i of covariate X (X_i indicates covariate value for individual i).

The differences in risk related to a specific covariate in this approach are estimated in terms of hazard ratio as follows:

$$HR = \frac{\hat{h}(t, X^*)}{\hat{h}(t, X)}$$

Equation 3.8

where X^* is the group with larger hazard and X is the group with smaller hazard for a specific covariate.

With one variable of interest ($X^* = 1, X = 0$) hazard ratio can be simplified to:[399]

$$HR = \frac{\widehat{h_0}(t)\,\exp(\sum_{i=1}^{p} \widehat{\beta_i} X_i^*)}{\widehat{h_0}(t)\,\exp(\sum_{i=1}^{p} \widehat{\beta_i} X_i)} = \exp\left(\sum_{i=1}^{p} \widehat{\beta_i}(X_i^* - X_i)\right) = e^{\widehat{\beta_i}}$$

Equation 3.9

Cox proportional hazards regression modelling has a number of advantages over logistic regression modelling or simple survival analysis. It does not have limitations of logistic models, which ignore survival times and censoring information, but unlike some of other maximum likelihood based approaches it does not make assumptions about the underlying baseline hazard or its distribution (it has a semi-parametric character).[400] It estimates the effect of a covariate in terms of change in hazard (hazard ratio) but because of the model form (baseline hazard multiplied by exponential of the effect) the estimated hazards are always non-negative. Hazard ratio itself is easily understandable and changes in hazard ratio are easy to interpret. It also allows for the non-linearity in the effect of continuous covariates, which can be explored graphically using splines or thought automated methods based on fractional polynomials.[401] A further advantage is that the model fit can be measured in terms of log likelihood or Akaike Information Criterion (AIC).

Efron approximation and Anderson-Gill repeated outcomes framework used in our analyses introduce further modifications to the basic Cox proportional hazards regression model allowing for recurrent events and reformulating of the problem as a counting process (taking place within specified time intervals).[380,402–404]

CHAPTER 4: THE IMPACT OF GENETIC POLYMORPHISMS ON THE PHARMACOKINETICS OF EFAVIRENZ IN AFRICAN CHILDREN.

4.1 Abstract

Aim: To characterise the efavirenz steady-state pharmacokinetics in African children using model-based approach, quantifying demographic and genotypic effects on the drug's disposition, and conduct simulations allowing prediction of optimised doses of efavirenz in this population.

Methods: We modelled the steady-state population pharmacokinetics of efavirenz in Ugandan and Zambian children using nonlinear mixed-effects modelling. Individual mid-dose efavirenz concentrations were derived and simulations explored genotype-based dose optimisation strategies.

Results: A 2-compartment model with absorption through transit compartments well described 2086 concentration-time points in 169 children. The combined effect of SNPs 516GT and 983TC explained 44.5% and 14.7% of the variability in efavirenz clearance and bioavailability, respectively. The detected frequencies of composite *CYP2B6* genotype were 0.33 for 516GG|983TT, 0.35 for 516GT|983TT, 0.06 for 516GG|983TC, 0.18 for 516TT|983TT, 0.07 516GT|983TC and 0.01 for 516GG|983CC. The corresponding estimated clearance rates were 6.94, 4.90, 3.93, 1.92, 1.36, and 0.74 L/h for a 15.4 kg child and median (95% CI) observed mid-dose concentrations 1.55 (0.51-2.94), 2.20 (0.97-4.40), 2.03 (1.19-4.53), 7.55 (2.40-14.74), 7.79 (3.66-24.59) and 18.22 (11.84-22.76) mg/L, respectively. Simulations showed that wild-type individuals had exposures at the bottom of therapeutic range, while slower metabolisers were over-exposed.

Conclusions: Dosage guidelines for African children should take into consideration the combined effect of SNPs *CYP2B6* 516G>T and 983T>C.

4.1.1 'What is known about this subject'

- High variability in efavirenz pharmacokinetics is largely contributed by SNPs in *CYP2B6:* 516G<T and 983T<C.
- SNP 983T<C is virtually absent in individuals of European ancestry.
- No previous studies quantified the effect of 983T<C on efavirenz clearance in children using model-based approach or recommended dose optimisation strategies accounting for this SNP.

4.1.2 'What this study adds'

- We propose a model concomitantly accounting for effect of weight and *CYP2B6* 516G<T|983T<C variants in African children.
- Using the model we simulated and compared exposures in weight-bands between *CYP2B6* metabolic subgroups and suggested a dose optimisation strategy adjusting for effect of both 516G>T and 983T>C.

4.2 Introduction

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) commonly used within first-line antiretroviral treatment (ART) for HIV-1 infected adults and children over 3 years.[11,63] Due to its ease of dosing (the long half-life allows once daily administration), proven efficacy, ability to be used with anti-TB drugs, and availability of cheap generic formulations, it is especially widely used in Africa.

Suboptimal efavirenz exposures have been previously related to treatment failure and high concentrations to central nervous system (CNS) side-effects.[69,94] Numerous studies reported very large between-subject variability (BSV) in efavirenz pharmacokinetics (PK).[74,94,168,221] This variability is attributed largely to single nucleotide polymorphisms (SNPs) in the *CYP2B6* gene which encodes the key metabolising enzyme. The loss-of-function polymorphism, 516G>T (rs3745274),[69,74,176,198] alters drug metabolism to the extent that dose adjustment based on *CYP2B6* 516G>T genotype is currently under investigation in children.[122,290] The proportion of slow metabolizers varies among different populations and is relatively high among black Africans.[69,76,176,198,221] In addition, efavirenz concentrations are affected by the functional polymorphisms *CYP2B6* 983T>C (rs28399499),[176,198] 785A>G (rs2279343)[39,203] and 15582C>T (rs4803419),[76,176] which are reported predominantly in black African and African-American patients; by polymorphisms involving its accessory pathways, including CYP2A6, CYP3A4 and UGT;[103,171,177] and in genes coding nuclear receptors CAR (NR1|3) and PXR (NR1|2), which regulate enzyme expression.[78,81] PK variability has also been linked to several physiological and environmental factors, such as sex,[36,39,221] ethnicity,[172,221,222] formulation type,[63,162] concomitant food[63] or co-medication (e.g. zidovudine[222], rifampicin and isoniazid[74,170,222]), and adherence[164]. However, reports on these effects have been to some extent contradictory and vary between adults and children.

Several investigators have reported a high proportion of sub-therapeutic efavirenz concentrations in children, highlighting the need for optimisation of paediatric dosing guidelines.[32,40,41] The aim of this analysis was therefore to characterise the steady-state PK of efavirenz in the largest cohort of African children reported so far, quantifying demographic and genotypic effects on efavirenz disposition, and thus allowing prediction of optimised doses of efavirenz in this population.

4.3 Methods

PK and other data from two studies in African children from Uganda and Zambia were pooled together: CHAPAS-3[382] (Children with HIV in Africa – Pharmacokinetics and Adherence/Acceptability of Simple antiretroviral regimens) and ARROW[41,381] (Anti-Retroviral Research for Watoto).

4.3.1 CHAPAS-3

Efavirenz was dosed once daily, *mane* or *nocte*, following modified WHO 2010 guidelines (Table 4.5), using a new paediatric double-scored 600 mg efavirenz tablet (provided by Cipla Pharmaceuticals, India) that can be split into 2 or 3 parts enabling administration of doses of 200, 300, 400, or 600 mg.

All included patients took part in sparse PK-sampling on clinic visits at week 6, 36, and every 24 weeks thereafter until the end of the study. The self-reported time of the last dose was recorded. Additional intensive PK-sampling was conducted in the first patients enrolled in each WHO weight-band at week 6 and in all patients who acquired tuberculosis during the study (4-10 weeks after tuberculosis treatment initiation and again 4-10 weeks after tuberculosis treatment cessation). Children in this intensive PK sub-study were advised to take efavirenz in the morning for 6 weeks prior to sampling and the drug intake on the PK day was observed. Plasma was separated and stored at −80°C until transportation on dry ice for drug concentration assay.

Plasma efavirenz concentrations from the intensive PK were assayed using ultra high-performance liquid chromatography at the Department of Clinical Pharmacy of Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands. The method produced linear results over the range of 0.0517 to 15.51 mg/L. The lower limit of quantification was 0.05 mg/L. The intraassay and interassay coefficients of variation (CV) were 1.01% to 5.31% and 0.1% to 1.63%, respectively. Relative error of the method ranged from 97.7% to 104.5%.[405] Plasma efavirenz concentrations from sparse PK were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) in the Division of Clinical Pharmacology, University of Cape Town, South Africa. The method was accurate over the range of 0.0195 to 20 mg/L. The lower limit of quantification was 0.0195 mg/L. The interassay coefficient of variation (CV) and residual error (RE) were 3.59%–5.78% and −2.57% to 0.92%, respectively, and the intraassay CV and RE were 1.50% to 9.67% and −6.00% to 4.28%, respectively.[71]

Both laboratories participate in international quality assurance and proficiency testing schemes and are expected to have comparable standards, although no cross-validation was performed for the assays. Systematic differences between the labs and assays were tested as covariates in the PK model (see Population Pharmacokinetic Analysis/Covariates).

4.3.2 ARROW

Efavirenz was dosed once-daily, *mane* or *nocte*, according to modified WHO 2006 paediatric recommendations. The tested formulations included 50, 100, and 200 mg capsules; and half or whole 600 mg tablets (provided by the national ART programme in Uganda).

Children in a PK sub-study[41] were sampled on 2 occasions: 36 and 40 weeks after starting ART. Eligible children were advised to take efavirenz in the morning for 4 weeks prior to sampling and drug intake on the PK day was observed. Samples were stored and assayed at the Department of Clinical Pharmacy of Radboud University Nijmegen Medical Centre, Nijmegen using the same methods as described above.

4.3.3 Genotyping

Genotyping was performed by allelic discrimination real-time PCR assay on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The PCR protocol involved an initial denaturation step at 95°C for 15 min, followed by 50 cycles of amplification at 95°C for 15 s and final annealing at 60°C for 1 min. TaqMan® Genotyping Master Mix and assays for *CYP2B6*-516G>T (rs3745274, C_7817765_60), *CYP2B6*-983T>C (rs28399499, C_60732328_20), *CYP2B6*-c.485-18C>T (rs4803419; C_7817764_10), *CYP2B6*-499C>G (rs3826711, C__27522377_10; ARROW patients only), *CYP3A4**22 (rs35599367, C__59013445_10), *CYP3A5*-6986G>A (rs776746, C__59013445_10), *NR1I3* (rs3003596, C__16194070_10; rs2307424, C__25746794_20), *NR1I2*-63396C>T (rs2472677, C__26079845_10) were obtained from Life Technologies Ltd (Paisley, Renfrewshire, UK). *CYP2B6**4 (785A>G, rs2279343) and *CYP2B6**29 copy number assay were performed on samples from ARROW study only using previously described custom TaqMan assays.[406,407] Opticon Monitor® version 3.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to obtain allelic discrimination plots and make allele calls. The assays were performed at the Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK.

The distribution of the genotypes was evaluated for compliance with Hardy-Weinberg equilibrium using the exact test conducted using R-package "genetics".[380]

4.3.4 Population pharmacokinetic analysis

4.3.4.1 Model building

The steady-state efavirenz PK was analysed using nonlinear mixed-effects modelling with software NONMEM VII (version 7.3)[408] and the first-order conditional estimation method with interaction. PsN 4.4.0, Pirana, and Xpose were used to facilitate modelling and for model diagnostics.[379] The model was developed and validated in accordance with standard methods described in the literature.[409] For the structural model, 1- , 2-, and 3-compartment models with first-order absorption and elimination were tested, as well as time lag or transit-compartment absorption[410] and hepatic first-pass model.[411] Between-subject and -occasion variability (BSV, BOV) were tested on PK parameters assuming lognormal distribution. Residual unexplained variability (RUV) was tested using a combined

proportional and additive error. Data below level of quantification (BLQ) were included in the analysis by imputing half of the LLOQ of the corresponding assay as suggested in Beal (M6 method).[412] Implausible samples and PK-profiles were identified using extreme values of CWRESI and their exclusion was evaluated based on visual checks.

Model development and covariate selection was guided by the NONMEM objective function value (OFV), inspection of goodness-of-fit (GOF) plots and visual predictive checks (VPCs), biological plausibility, and clinical relevance. OFV (proportional to -2 log-likelihood of the data) was assumed to be χ^2-distributed and a drop of 3.84 or more between two hierarchical models after inclusion of one additional parameter (df=1) was considered a significant improvement (p=0.05). Stability and robustness of the final model, together with precision of its parameter estimates, was evaluated through a nonparametric bootstrap (n=200).

Intensive and sparse data were included in the model development process in a stepwise manner as suggested in Svensson *et al.,*[294] starting with intensive PK data from CHAPAS-3, followed by the intensive data from ARROW, and finally the sparse PK data from CHAPAS-3.

The model-derived Empirical Bayesian Estimates for the individual parameters were used to predict steady-state mid-dose concentrations (measures 12h after dose) for each sampling occasion and patient.

4.3.4.2 Covariates

Allometric scaling was added to the model at an early development stage as previously suggested.[225] The effect of maturation of metabolic pathways on PK parameters was tested using post-menstrual age (gestation-adjusted age) as a predictor. Both a power function or a sigmoidal model with and without Hill coefficient were tested.[225] Besides weight and age, the other covariates tested were: tuberculosis co-treatment, study site, nucleoside reverse transcriptase inhibitor (NRTI) backbone, sex, weight-for-age Z-score (WAZ) and height-for-age Z-score (HAZ), drug formulation, the effect of splitting tablets used in CHAPAS-3 (inferred from total daily dose), and genotyping information (SNPs listed above). The potential difference between assays and lab procedures for the quantification of drug concentrations was tested in the model as proportionality and correction factors on RUV.

Missing genotype values were imputed using mixture modelling with frequencies fixed to those observed in the rest of the cohort as previously suggested by Keizer et al.[413]

4.3.4.3 Simulations

The final model was used to simulate exposures after administration of efavirenz with the formulation given in CHAPAS-3 and using a dataset of subjects with a uniform distribution of weights ranging from

10 to 40 kg, in 0.1 kg steps (300 individuals simulated 100 times). Several dosing strategies were explored. To avoid generating implausibly extreme values, the maximum variability for each random effect was limited to 3 standard deviations. Data were analysed and plots generated using R.[380]

4.4 Results

4.4.1 Demographic results and samples

This analysis included data from 128 children from CHAPAS-3, and 41 children from ARROW. Relevant subject characteristics including the genotype frequencies for the tested SNPs are presented in Tables 4.1 and 4.2. All tested genotypes were in Hardy-Weinberg equilibrium (HWE). For SNPs rs35599367, rs3826711 and *CYP2B6**29 all patients were homozygous for the common allele and HWE was not calculated. The genotype information was missing for 5 children from ARROW and 2 from CHAPAS-3, who were assigned by the mixture model as follows: 2 as 516GG|983TT, 4 as 516GT|983TT, and one as 516TT|983TT.

Table 4.1 Demographic characteristics of children in ARROW and CHAPAS-3 treated with efavirenz (part 1)

Characteristics	ARROW iPK	CHAPAS-3		Combined
		iPK	sPK	
No. of children*	41	51	128	169
No. of samples	611	474	1002	2087
Sampling schedule	0h, 1h, 2h, 4h, 6h, 8h, 12h, 24h		2 samples 2 h apart	
No. of samples excluded	9	8	5	22
Age [years]**	7.6 (4.0-12.5)	4.5 (2.1-13.8)		4.7 (2.1-13.8)
Weight [kg] **	20.0 (14.0-30.0)	15.0 (7.8-29.9)		15.5 (7.8-30.0)
Sex [M/F]	17/24	63/65		80/89
Race	Black African			
CYP2B6 516GT (rs3745274; HWE p=1)†				
GG	16 (44%)	49 (39%)		65 (40%)
GT	14 (39%)	53 (42%)		67 (41%)
TT	6 (17%)	24 (19%)		30 (19%)
MAF	0.36	0.40		0.39
CYP2B6 983T>C (rs28399499; HWE p=0.6)†				
TT	33 (92%)	106 (84%)		139 (86%)
TC	3 (8%)	19 (15%)		22 (14%)
CC	0 (0%)	1 (1%)		1 (1%)
MAF	0.04	0.08		0.07

Note: explenations below Table 4.2

Table 4.2 Demographic characteristics of children in ARROW and CHAPAS-3 treated with efavirenz (part 2)

Characteristics	ARROW	CHAPAS-3	Combined
CYP2B6 15582C>T (rs4803419; HWE p=1)†			
CC	32 (89%)	113 (90%)	145 (90%)
TC	4 (11%)	13 (10%)	17 (10%)
MAF	0.06	0.05	0.05
CYP3A4*22 (rs35599367)†			
GG	36 (100%)	126 (100%)	162 (100%)
CYP3A5 6986G>A (rs776746; HWE p=0.57)†			
GG	1 (3%)	2 (2%)	3 (2%)
GA	7 (19%)	41 (33%)	48 (30%)
AA	28 (78%)	83 (66%)	111 (69%)
MAF	0.12	0.18	0.17
NR1I3 (rs3003596; HWE p=0.34)†			
AA	7 (19%)	30 (24%)	37 (23%)
AG	18 (50%)	55 (44%)	73 (45%)
GG	11 (31%)	41 (33%)	52 (32%)
MAF	0.46	0.46	0.46
NR1I3 540 C>T (rs2307424; HWE p=1)†			
TT	0	1 (1%)	1 (1%)
CT	3 (8%)	23 (18%)	26 (16%)
CC	33 (92%)	102 (81%)	135 (83%)
MAF	0.04	0.10	0.09
NR1I2 63396C>T (rs2472677; HWE p=0.07)†			
CC	13 (36%)	43 (34%)	56 (35%)
CT	16 (44%)	72 (57%)	88 (54%)
TT	7 (19%)	11 (9%)	18 (11%)
MAF	0.42	0.37	0.38
CYP2B6*4 785A>G (rs2279343; HWE p=0.47)‡			
AA	16 (44%)	not tested	
AG	14 (39%)		
GG	6 (17%)		
MAF	0.36		
CYP2B6 499C>G (rs3826711)‡			
CC	36 (100%)	not tested	
CYP2B6*29‡			
***1/*1**	36 (100%)	not tested	

Note: Data are median (range) or no. (%) of subjects; *51 children in the CHAPAS-3 study who underwent both intensive and sparse sampling are counted in both categories; **Baseline values; †162 pts from both CHAPAS-3 and ARROW studies; ‡36 pts from ARROW study; iPK – intensive sampling; sPK – sparse sampling; HWE - Hardy-Weinberg equilibrium, MAF – minor allele frequency

From CHAPAS-3, 61 intensively sampled PK-profiles (a total of 474 samples) and 510 sparse PK-profiles (1002 samples, 1-2 per occasion) were available. The PK data were collected from 6 weeks after starting efavirenz up to a maximum of 132 weeks. There were up to 7 PK-sampling visits per child. Of 14 children who acquired tuberculosis, 9 had at least one intensively sampled PK-profile on efavirenz with tuberculosis treatment. The only BLQ measurement from intensive PK sub-study was a pre-dose measurement and all 8 samples from that PK visit for that patient were excluded from the analysis as it was deemed likely not to be in steady-state due to poor adherence. Within the sparse data, 15 samples were BLQ and were included by imputing half LLOQ, i.e. 0.00975 mg/L. From the ARROW study, 611 intensive PK samples from 82 PK visits (2 visits per patient) were available. Data from one visit were discarded due to an implausible PK profile, possibly caused by mismatch of samples. No samples were BLQ.

4.4.2 Population pharmacokinetics

The data were best described using a 2-compartment model with first-order elimination and transit compartment absorption.[410] Final parameter estimates, their precision (obtained through a bootstrap) and statistical significance for the inclusion of the covariate and random effects (based on drop in OFV) are presented in Table 4.3. The PK parameters were estimated relative to oral bioavailability whose typical value was fixed to 1 due to lack of intravenous data. Adequate fit of the model was confirmed by a GOF plots and VPC (Figures 4.4 and 4.5 in Appendix to Chapter 4).

The effect of body size on all clearance and volume parameters was accounted for using allometric scaling, which significantly improved model fit (18 points drop in OFV).[225] No effect of age on the maturation of clearance could be detected. After adjusting for body size, the main predictor of clearance was the effect of *CYP2B6* genotype, categorised into 6 subgroups based on the combined effect of 516G>T and 983T>C SNP-variants present in our population (Table 4.3 and 4.4). *CYP2B6* genotype explained 44.5% and 14.7% of BSV in clearance and oral bioavailability respectively. Exclusion of individuals with missing genotype did not have a significant effect on final results.

The absorption rate constant (k_a) and the absorption mean transit time (MTT) were 1.6-fold larger and 1.4-times longer in ARROW compared with CHAPAS-3. Splitting of the new double-scored efavirenz tablets used in the CHAPAS-3 was not found to affect efavirenz bioavailability. No other covariate (see Methods) was found to significantly improve the model fit. We did not detect any systematic differences between the assays and labs employed in the analysis.

The model fit was markedly improved by inclusion of a correction parameter to allow for larger residual unexplained variability for all samples obtained after self-recorded efavirenz intake. This

*Table 4.3 Final efavirenz population parameter estimates (5th and 95th percentile)**

. Fixed Effects (THETA)			p-value†	Random Effects (ETA)**		p-value†
F		1 (FIXED)		BSVBIO	42.2% (31.3%-50.8%)	p<0.001 (dOFV=19.7, df=1)
				BOVBIO	50.5% (42.3%-55.9%)	p<0.001 (dOFV=304.3, df=1)
NN [number]		25.0 (17.7-35.1)				
MTT [h]	CHAPAS-3	0.82 (0.69-0.96)	p<0.001 (dOFV=21.4, df=1)	BOVMTT	78.0% (71.5%-96.1%)	p<0.001 (dOFV=443.3, df=1)
	ARROW	1.17 (1.02-1.37)				
Ka [1/h]	CHAPAS-3	0.79 (0.37-0.95)	p<0.001 (dOFV=37.9, df=1)	BOVKA	57.7% (45.5%-69.0%)	p<0.001 (dOFV=96.7, df=1)
	ARROW	1.27 (0.90-1.62)				
CL [L/h]	516GG|983TT	6.94 (6.47-7.61)	p<0.001 (dOFV= 154.7, df=5)	BSVCL	36.9% (24.9%-45.6%)	p<0.001 (dOFV=64.2, df=1)
	516GG|983TC	3.93 (2.61-5.65)				
	516GG|983CC	0.74 (0.72-0.75)		BOVCL	26.6% (18.9%-35.4%)	p<0.001 (dOFV=26.9, df=1)
	516GT|983TT	4.90 (4.40-5.46)				
	516GT|983TC	1.36 (0.97-1.76)				
	516TT|983TT	1.92 (1.52-2.33)		Error Model		
Vc [L]		64.1 (49.1-73.3)		Additive error [mg/L]	0.101 (0.067-0.131)	p<0.001 (dOFV=199.2, df=1)
Q [L/h]		17.1 (14.1-20.9)		Proportional error [%]	0.0672 (0.052-0.079)	p<0.001 (dOFV=678.5, df=1)
Vp [L]		92.2 (80.1-112.7)		Increased error for sparse data	2x (1.7x -2.5x)	p<0.001 (dOFV=17.7, df=1)

Note: Final parameter estimates are typical population values estimated by the model. All clearance and volume parameters scaled allometrically to median weight of 15.4kg. *Estimated from nonparametric bootstrap (n=200) of the final model; **Expressed as approximate %CV on SD scale ($\sqrt{ETA}$*100). †Change in the objective function value after elimination of the parameter from the final model (dOFV > 10.83 corresponds to p < 0.001). F – bioavailability; NN – number of transit compartments; MTT – mean transit time; Ka – absorption rate constant; CL – clearance; Q – inter-compartmental clearance; Vc – volume of central compartment; Vp – volume of peripheral compartment; BSVBIO – between subject variability in bioavailability; BOVBIO - between occasion variability in bioavailability; BOVMTT - between occasion variability in medium transit time; BOVKA - between occasion variability in absorption rate constant; BSVCL - between subject variability in clearance; BOVCL - between occasion variability in clearance.

includes all pre-dose intensive PK-samples and all the sparse PK-samples from CHAPAS-3. The residual variability for those samples was twice as large as in the rest of the data

4.4.3 Simulations

Simulations were performed to predict exposures in African children based on their weight and genotype, when dosed according to the regimen used in CHAPAS-3. Median mid-dose concentrations were comparable across weight-bands but noticeable differences were observed between the *CYP2B6* genotype subgroups (Figure 4.1, Table 4.4).

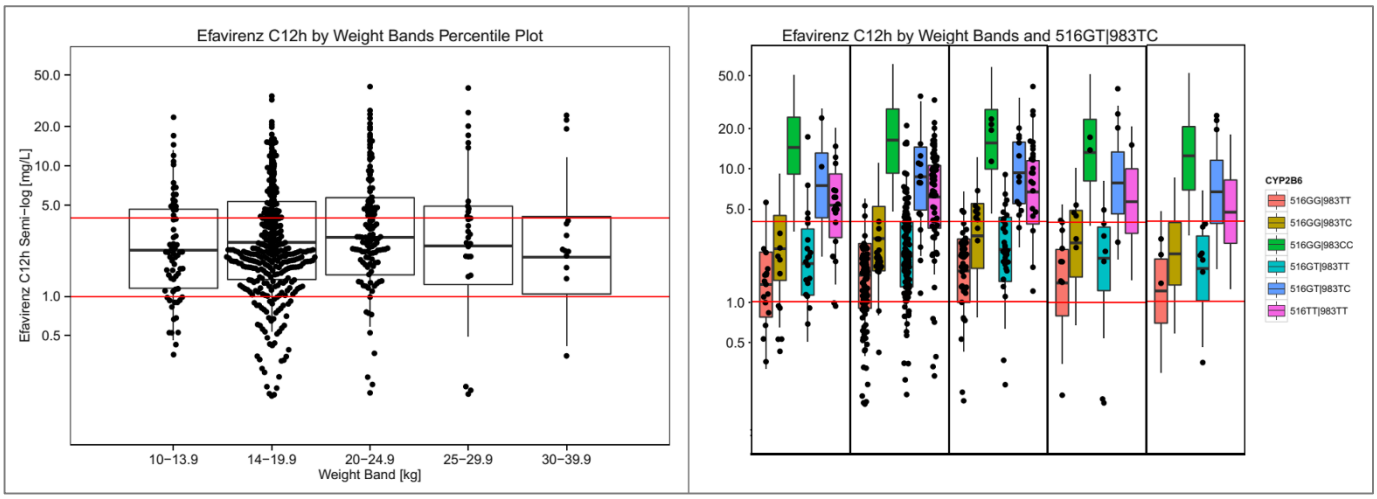

Figure 4.1 Individual efavirenz mid-dose concentrations estimated by the population pharmacokinetic model and the simulated mid-dose concentrations across weight-bands and by different CYP2B6 516GT|983TC subgroups

Note: Individual mid-dose concentrations estimated by the population pharmacokinetic model (black dots) are plotted on top of the mid-dose concentrations (percentile plots) simulated across weight-bands (left) and by different CYP2B6 516GT|983TC subgroups (right). Red horizontal lines correspond to efavirenz concentrations of 1mg/L and 4mg/L.3 Breaks in the percentile plot correspond to 25th, median and 75th percentile and whiskers correspond to 5th and 95th percentile of the simulated data.

A dose optimisation strategy for African children was devised by categorising subjects into four phenotypic subgroups based on their composite genotype vector 516G>T|986T>C, similarly to Dooley *et al.*[71] (presented in Table 4.4). The proposed dose-adjustment between metabolic subgroups is based on optimal ratios of 1:0.66:0.33:0.1 for EM:IM:SM:USM (extensive, intermediate, slow and ultra-slow metabolisers), respectively, and is outlined in Table 4.5.

The predicted exposures based on the suggested dose-optimisation algorithm are presented in Figure 4.2 and Table 4.6 (in the Appendix to Chapter 4). The suggested dosing approach ensured adequate drug exposure in all simulated weight-bands (Figure 4.2, left panel), and reduced the differences due to metabolic status (Figure 4.2, right panel).

Table 4.4 Efavirenz PK exposures of different metabolic subgroups determined by 516GT|983TC SNP vector

SNP Vector	MET	Pts*	CL [L/h]*	C12h [mg/L]**†	C12h < 1 [mg/L]**	1> C12h <4 [mg/L]**	C12h > 4 [mg/L]**	C24h [mg/L]**†	AUC [mg·h/L] **†
516GG|983TT	EM	56 (33.1%)	6.94	1.55 (0.51-2.94)	40 (22%)	132 (74%)	6 (3%)	0.86 (0.26-2.02)	37.53 (14.26-75.12)
516GG|983TC	IM	10 (5.9%)	3.93	2.03 (1.19-4.53)	7 (16%)	28 (62%)	10 (22%)	1.33 (0.65-3.66)	46.30 (30.65-118.08)
516GG|983CC	USM	1 (0.6%)	0.74	18.22 (11.84-22.76)	0 (0%)	0 (0%)	6 (100%)	17.28 (11.20-21.63)	438.94 (286.10-548.20)
516GT|983TT	IM	59 (34.9%)	4.90	2.20 (0.97-4.40)	19 (10%)	132 (69%)	40 (21%)	1.54 (0.58-3.54)	56.05 (25.16-105.47)
516GT|983TC	SM	12 (7.1%)	1.36	7.79 (3.66-24.59)	0 (0%)	6 (17%)	29 (83%)	6.97 (3.24-23.07)	258.42 (64.81-548.77)
516TT|983TT	SM	31 (18.4%)	1.92	7.55 (2.40-14.74)	7 (6%)	20 (18%)	82 (75%)	6.61 (1.93-13.35)	175.98 (49.61-356.44)

Note: Data are population median (5^{th}-95^{th} percentile) or number (percentage). Based on all patients from ARROW and CHAPAS-3 trials (missing genotype estimated by mixture model). *CL refers to typical population value estimated by the model for a patient with median weight of 15.4 kg (combined ARROW and CHAPAS-3 data). **CHAPAS-3 data only (due to differences in dosing between studies, see Methods). Value for each PK visit estimated by the model, multiple measurements used to calculate geometric mean for every patient which were then used to calculate median and percentiles for each subgroup. MET – metabolic subgroup:[71] EM (extensive metabolisers) - 516GG|983TT; IM (intermediate metabolisers) - 516GG|983TC or 516GT|983TT, SM (slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (ultra-slow metabolisers) - 516GG|983CC. The light grey shading indicates groups of patients who would be significantly overexposed if dose optimisation would be conducted based only on SNP 516G>T.

4.5 Discussion

Efavirenz pharmacokinetics in Africans has been previously shown to be affected by the combined effect of SNPs 516G>T and 983T>C.[71,76,103,176,203] The current investigation confirms those findings and is the first analysis to quantify the effect of the *CYP2B6* 516G>T|983T>C SNP-vector on efavirenz clearance in African children using nonlinear mixed-effects modelling. The use of modelling provides a tool to concomitantly account for multiple effects such as genotype and weight, and a platform to derive a dose adjustment strategy based on these effects.

Numerous studies in adults and children have reported a significant effect of *CYP2B6* 516G>T on efavirenz clearance. Our analysis shows that presence of one variant allele in 516G>T causes clearance to drop by 34%, while the reduction reaches 72% for homozygous mutants, which is in line with previously reported reductions of 20-47% and 58%-80%, respectively.[34,36,39,40,169,170,173] Our findings also show that the effect of SNP 516G>T is significantly modified by the 983T>C (i.e. in wild type 516G>T individuals presence of a single variant allele in 983T>C causes a 43% drop in clearance and 89% if no functional allele is present), confirming associations found by *Holzinger et al*.[176] and in a number of African studies.[71,76,203] This polymorphism is virtually absent in individuals of European ancestry[176,198] and was not detected in a study of Cambodian patients.[170] The combined effect of the *CYP2B6* 516G>T|983T>C SNP-vector on efavirenz clearance has been previously reported distinguishing 4 phenotypic subgroups.[71,103] In our study, we were able to further characterise this effect using six *CYP2B6* 516G>T|983T>C variant subgroups. Similarly to previous reports we show that, despite its low prevalence, SNP 983T>C is not only a significant predictor of efavirenz clearance, but it is responsible for a larger drop in metabolic rate than 516G>T (i.e. 29% drop in clearance in 516G>T heterozygote vs 43% drop in 983T>C heterozygote, when no other polymorphisms were present).[76,176] No further significant genetic associations were detected.

A genome-wide association study by Holzinger *et al*. identified rs4803419 as another significant polymorphism in *CYP2B6* affecting efavirenz clearance.[176] The effect of SNP rs4803419 becomes significant only for homozygous mutants who are wild type for 516G>T and 983T>C and no such variants were present in our population. Although this finding was recently replicated in South African patients, the investigators concluded this effect was negligible in comparison to 516G>T and 983T>C.[76]

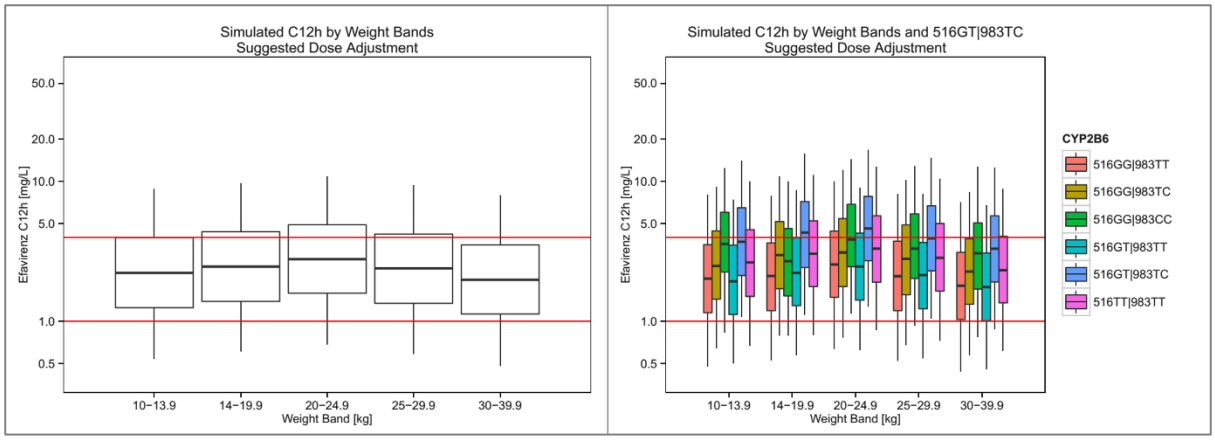

Figure 4.2 Simulated efevirenz mid-dose concentrations across weight-bands and by different 516GT|983TC genotypes based on suggested most optimal dosing.

Note: The left plot shows the simulated efevirenz mid-dose concentrations across weight-bands and the right one by different 516GT|983TC genotypes based on suggested most optimal dosing. Red horizontal lines correspond to efavirenz concentrations of 1mg/L and 4mg/L.[94] Breaks in the percentile plot correspond to 25th, median and 75th percentile and whiskers correspond to 5th and 95th percentile of the simulated data

Results from the simulations (Figure 4.1) showed that, even though the dosage guidelines tested in CHAPAS-3 result in average mid-dose concentrations within target range of 1.0-4.0 mg/L,[94] the effect of *CYP2B6* genotype leads to large differences within each weight-band. In particular, children with slower *CYP2B6* genotypes (516GG|983CC, 516GT|983TC, and 516TT|983TT) were over-exposed, while the fastest metabolisers (516GG|983TT) achieved exposures at the bottom of the therapeutic range. Over 20% of children in the study with the 516GG|983TT genotype had efavirenz concentrations below the proposed minimum target concentration of 1.0 mg/L (Table 4.4). Moreover, our model indicates that disregarding the effect of the 983T>C SNP and basing dose optimisation only on 516G>T could lead to exposures significantly higher than the therapeutic range[94] in ~14% of African patients with 983TC or 983CC genotypes (Table 4.4 in grey). This suggests that genotype-based dose optimisation in African children should take into account both 516G>T and 983T>C SNPs.

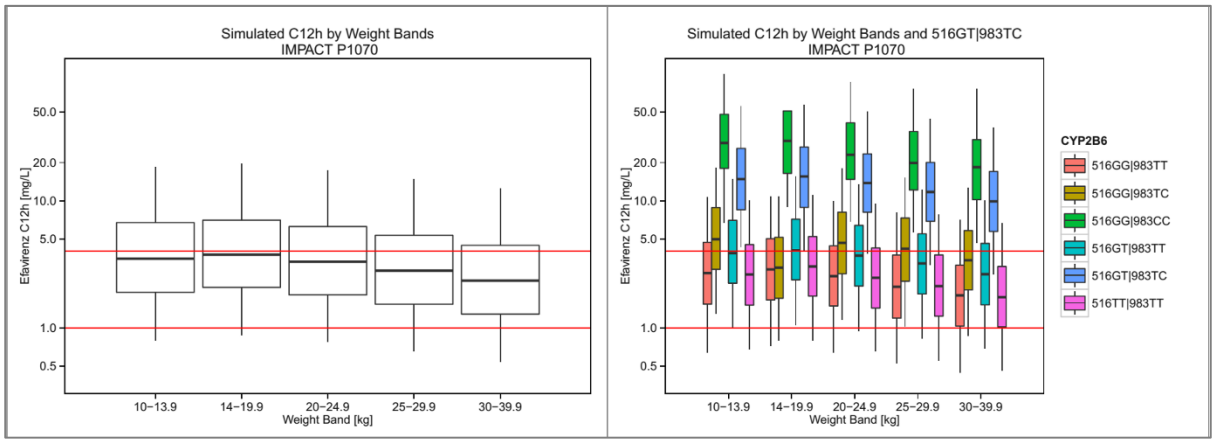

Figure 4.3 Simulated efavirenz mid-dose concentrations across weight-bands and by different 516GT|983TC genotypes based on dose recommendations tested in IMPACT study P1070 applied to our population.

Note: The left plot shows the simulated efavirenz mid-dose concentrations across weight-bands and the right one by different 516GT|983TC genotypes based on dose recommendations tested in IMPACT study P1070 applied to our population. Red horizontal lines correspond to efavirenz concentrations of 1mg/L and 4mg/L.[94] Breaks in the percentile plot correspond to 25th, median and 75th percentile and whiskers correspond to 5th and 95th percentile of the simulated data.

The only available guidelines on genotype-adjusted paediatric dosage were recently formulated for patients under 3 years by the Panel on Antiretroviral Guidelines for Adults and Adolescents at the Department of Health and Human Services (dHHS) and are currently being tested in the IMPAACT study P1070.[122,290] The dose adjusted strategy was developed based on results of an analysis by Salem *et al*.[162] and preliminary results of IMPAACT P1070[289] and proposed different dosing for individuals with 516GG or 516GT genotype versus 516TT. In contrast to previous paediatric studies,[34,75] Salem *et al*. did not detect significant differences in clearance rate between patients with 516GG and 516GT genotypes, and the effect of 983T>C was not evaluated. Their findings might differ from the current investigation for several reasons: the study by Salem *et al.* included a smaller number of patients, the lowest age of participants was only 2 months, the tested population comprised patients of various races and the formulations included capsules and liquid. According to simulations from the current model, direct application of that strategy in African children could result in result in exposures above the therapeutic range[94] in a large proportion of patients who are either heterozygous for 516G>T or wild type with 983TC or 983CC genotypes (Figure 4.3).

Table 4.5 Efavirenz dosage tested in CHAPAS-3 vs. proposed genotype adjusted dose optimisation

CHAPAS-3		Based on 516GT|986TC				
Weight [kg]	Dose	Weight [kg]	EM	IM	SM	USM
			1	0.66	0.33	0.1
10 – 13.9	200	**10 – 13.9**	300	200	100	50
14 – 19.9	300	**14 – 19.9**	400	300	150	50
20 – 24.9	400	**20 – 24.9**	600	400	200	100
25 – 29.9	400	**25 – 29.9**	600	400	200	100
30 – 39.9	400	**30 – 39.9**	600	400	200	100

Note: EM (extensive metabolisers) - 516GG|983TT; IM (intermediate metabolisers) - 516GG|983TC or 516GT|983TT, SM (slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (ultra-slow metabolisers) - 516GG|983CC.[71] The dose recommendations were rounded to the nearest full entity of currently available formulations (50 mg capsule, 100 mg capsule and 600 mg double scored tablets allowing dose of 200 mg, 300 mg, 400 mg and 600 mg)

Our recommendations were simplified to previously described 4 metabolic subgroups determined by the composite 516G>T|983T>C vector outlined in Table 4.4.[71] The clearance between EM:IM:SM:USM drops as follows: 1:0.6:0.24:0.1, which is remarkably similar to the ratios detected by *Dooley et al*. in African adults (1:0.6:0.26:0.08, respectively).[71] Our dosage algorithm presented in Table 4.5 (1:0.66:0.33:0.1) was adjusted to maximise the use of currently available solid formulations and conducted simulations show it would provide optimal drug exposures across all weight bands acounting adequately for individual *CYP2B6*516G>T|983T>C genotype (Figure 4.2). A similar dose adjustment pattern (1:0.66:0.33) was previously successfully implemented based of phenotypic differences in an adult study by Mello *et al.*[261]

The results of the few dose reduction studies guided by *CYP2B6* genotype conducted in developed countries highlighted improved treatment tolerability and cost-effectiveness in adults.[258,259,261,414] These results were confirmed by recent cost-effectiveness analysis of this practice in American adults.[263] It could be speculated that in a resource-limited settings, cost and logistical challenges would make implementation of such practice difficult. Nonetheless, decreasing price and broader availability

of genotyping technology and economic development open future scenarios in which genotype-based dosing approaches may be economically viable and beneficial even in developing countries. The IMPAACT P1070 study, which is currently being conducted in HIV-infected infants and children, should hopefully give more insight into practical implications of genotyping in low- and middle-income countries.

Similarly to previous paediatric studies, the average clearance value (before inclusion of genotype effect) was higher than findings in adults - 14.34 L/h versus 7.5 to 11.7 L/h (both after scaling with allometry up to 70 kg).[34,168–170,172] This is consistent with reports that efavirenz clearance in children 1-4 years exceeds adult values.[32,34,277] In keeping with previously published paediatric models, the effect of size on clearance and volume was explained through allometric scaling.[34,162,225] Unlike Salem *et al*. we did not observe age-related maturation of clearance, but the previous analysis showed that 90% of maturation was reached by the age of 9 months[162] and the majority of patients in the current study were >3 years.

We detected significant differences in absorption parameters between CHAPAS-3 and ARROW, possibly related to the use of different formulations (tablets in former and mostly capsules in latter). The formulations were assumed to be bioequivalent and indeed no formulation effect on bioavailability was detected.

Due to the availability of data from multiple sampling occasions within the same patient, it was possible to characterise both BSV and BOV in the PK parameters. Large BOV was identified for absorption parameters and bioavailability. Drug absorption is widely known to be a variable phenomenon, depending on occasion-specific factors, such as food intake, gastric emptying times, and GI-tract pH. In the current study, other factors may have contributed to inflating BOV (in particular on bioavailability), including differences between actual and self-reported intake times for the sparse data, lack of accurate intake history before the last dose, lack of information on accompanying food consumption. The fact that the information about intensively sampled occasions was more accurate was accounted for with the introduction of a scaling factor on residual unexplained variability (2-fold larger for sparse data).

The current study had several limitations. As mentioned, the dosage timing and food co-administration was not recorded beyond the last intake. Previous studies showed that efavirenz PK was affected by adherence and food effects.[63,164,172] Polymorphisms in accessory pathways including CYP2A6 and CYP3A4 or UGT were not assessed and the effect of *CYP2B6* 785A>G was only evaluated in patients from the ARROW study. Despite several reports suggesting that efavirenz metabolism is

affected by polymorphisms in those pathways, the genome-wide association study by Holzinger *et al.* showed that their effect was significantly less dramatic than for 516G>T and 983T>C.[176]

Furthermore, no PK/PD relationship was explored in this analysis for either efficacy or toxicity, but currently accepted therapeutic ranges were used as cut-offs guiding dose optimisation. These targets were generated in an adult cohort and have recently been brought into question suggesting that lower efavirenz concentrations might be sufficient to provide viral suppression,[103] however no alternative has been suggested to date.

Lastly our study was underpowered to determine the effect of tuberculosis treatment on efavirenz concentrations, but recent findings suggest that the inducing effect of rifampicin on clearance is counterbalanced by a concentration-dependent inhibitory effect of isoniazid that could explain contradictory conclusions from previous studies.[71,74,170]

4.6 Conclusions

Our study suggests that genotype-adjusted efavirenz dosage in African children should be based on the composite 516G>T|983T>C SNP-vector, due to significant modification of clearance rates caused by SNP 983T>C genotype, whose prevalence in Africans is much higher than other populations. Using nonlinear mixed-effects modelling we quantified this effect and suggest that a dose optimisation algorithm 1:0.66:0.33:0.1 (EM:IM:SM:USM, respectively) would provide more balanced drug exposures between individuals with a different metabolic status while maximising the potential of using the new double-scored efavirenz tablets tested in the CHAPAS-3 study. The findings warrant further studies evaluating the genotype-based dosing approach and the feasibility of genotyping in resource-limited settings.

4.7 Conflict of interest

All authors have completed the Unified Competing Interest form and declare: AB, AC, VM, CK, AD, ASM, DMG, HM and DB received support through grants from European Developing Countries Clinical Trials Partnership (EDCTP); AC, AD, ASM and DMG additionally received grants from Medical Research Council (MRC) UK; HM additionally declares support in part by the National Research Foundation of South Africa, grant 90729; AO received support in form of grants from Janssen, ViiV and Tandem Nano, as well as personal fees from Merck was issued a patent “Compositions of efavirenz”. No other support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work are to be declared for any of the authors.

4.8 Funding Statement

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CHAPAS-3 was funded by EDCTP (IP.2007.33011.006), MRC UK, Department for International Development UK, Ministerio de Sanidad y Consumo Spain. Cipla Ltd donated first-line antiretrovirals.

The drug assays were supported in part by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (UM1 AI068634, UM1 AI068636 and UM1 AI106701, U01 AI068632), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and the National Institute of Mental Health (AI068632). HM is supported in part by the National Research Foundation of South Africa, grant 90729. The content is solely the responsibility of the authors and does not necessarily represent the official views of any funders.

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The Division of Clinical Pharmacology at the University of Cape Town would like to gracefully acknowledge Novartis Pharma for their support of the development of pharmacometrics skills in Africa.

4.10 APPENDIX TO CHAPTER 4

4.10.1 Supplementary figures

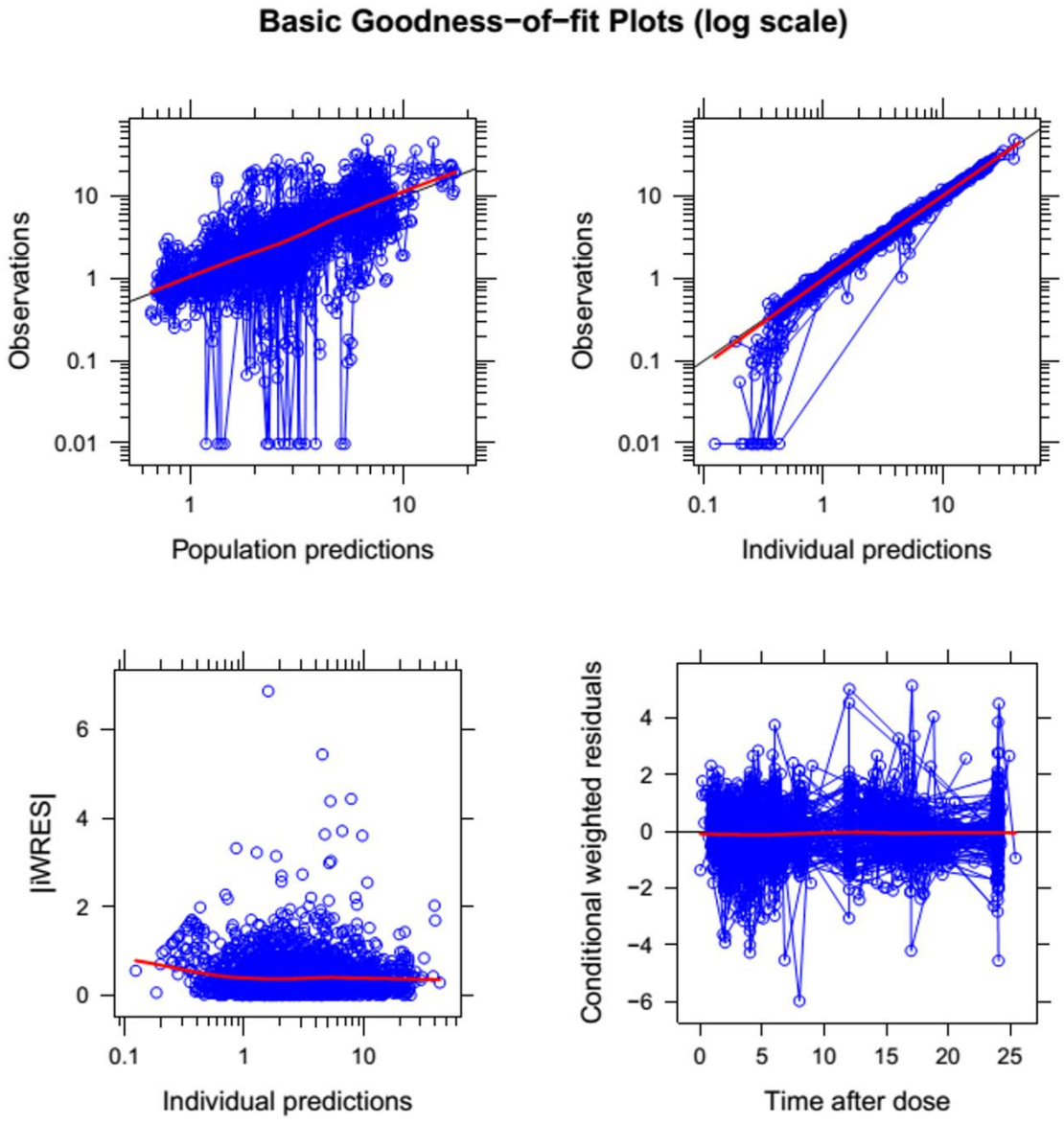

Figure 4.4 Goodness of fit plots for the final efavirenz population pharmacokinetic model

Note: Top left – observations vs population predictions; top right – observations vs individual predictions; bottom left – conditional weighted residuals vs time after dose; bottom right – absolute values of individual weighted residuals vs individual predictions

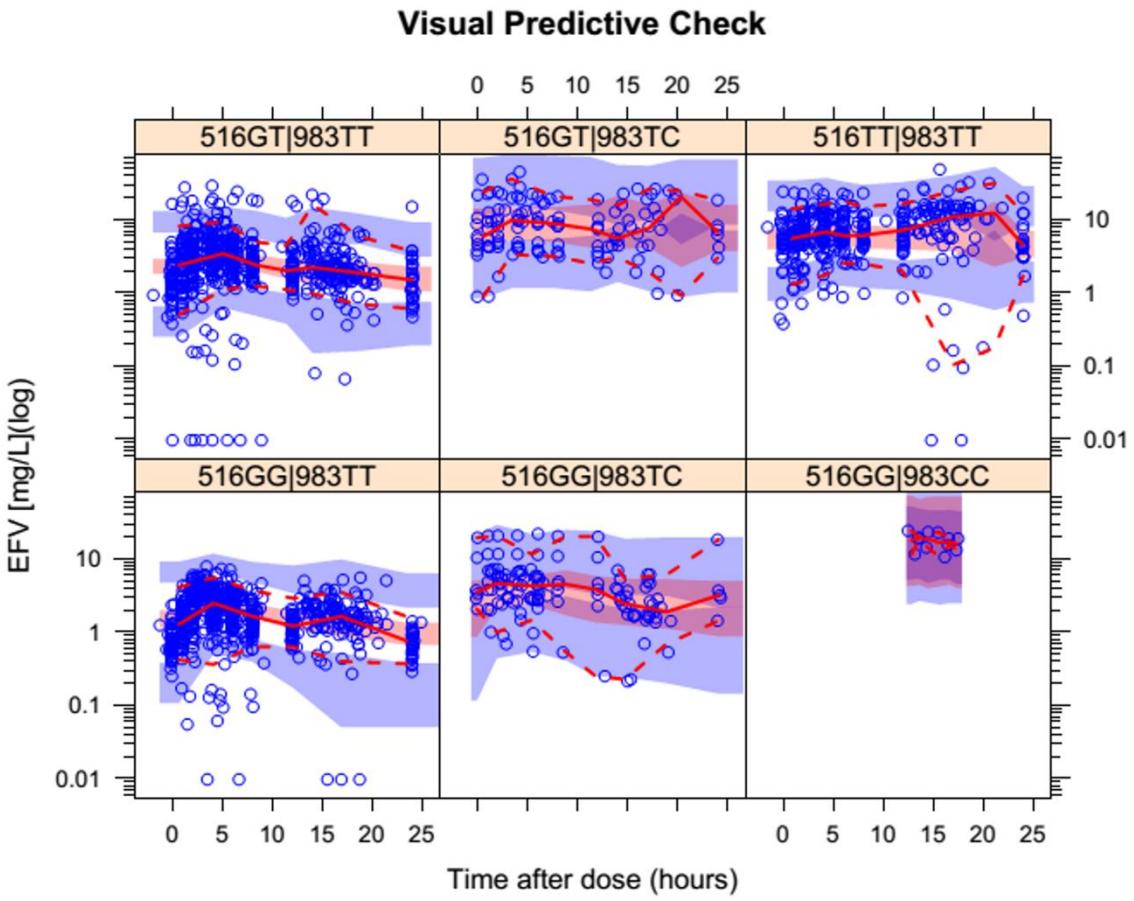

Figure 4.5 Visual Predictive Check of the final efavirenz population pharmacokinetic model by 516G>T|983T>C SNP vector in semi-log scale.

Note: Hollow points – observations, red solid line – median of observed data, red line with breaks – 5th and 95th percentile of observed data, orange fill area – 95% confidence interval of simulated median, blue fill area - 95% confidence interval of simulated 5th and 95th percentile

4.10.2 Supplementary tables

Table 4.6 Simulated values of efavirenz mid-dose concentrations obtained under various dosage scenarios and proportions of patients <1mg/L, 1mg/L>&<4mg/L and >4mg/L

Weight Band [kg]	Metabolic group	FM	IM		SM		USM
	SNP Vector	516GG|983TT	516GG|983TC	516GT|983TT	516GT|983TC	516TT|983TT	516GG|983CC
10-13.9	Dose [mg]	300	200		100		50
	C12h [mg/L]	2.02 (0.47-8.04)	2.49 (0.64-9.11)	1.93 (0.50-7.42)	3.71 (1.08-14.01)	2.63 (0.67-10.04)	3.57 (0.83-12.43)
	>4 [mg/L]	21%	29%	20%	46%	30%	45%
	1> & <4 [mg/L]	60%	58%	59%	50%	57%	49%
	<1 [mg/L]	20%	13%	21%	4%	12%	7%
14-19.9	Dose [mg]	400	300		150		50
	C12h [mg/L]	2.11 (0.52-7.86)	2.98 (0.79-10.90)	2.22 (0.57-8.68)	4.30 (1.10-15.77)	3.04 (0.79-11.12)	2.70 (0.79-9.99)
	>4 [mg/L]	22%	36%	24%	53%	37%	30%
	1> & <4 [mg/L]	60%	55%	59%	43%	54%	60%
	<1 [mg/L]	19%	9%	16%	4%	9%	10%
20-24.9	Dose [mg]	600	400		200		100
	C12h [mg/L]	2.55 (0.63-9.98)	3.11 (0.76-12.07)	2.47 (0.62-8.98)	4.6 (1.27-16.79)	3.31 (0.87-12.68)	3.84 (1.13-14.30)
	>4 [mg/L]	29%	38%	28%	57%	41%	47%
	1> & <4 [mg/L]	58%	54%	58%	40%	52%	48%
	<1 [mg/L]	13%	8%	14%	3%	7%	4%
25-29.9	Dose [mg]	600	400		200		100
	C12h [mg/L]	2.10 (0.52-8.12)	2.80 (0.67-10.20)	2.14 (0.54-8.14)	3.92 (1.04-14.74)	2.84 (0.73-10.42)	3.31 (0.93-12.77)
	>4 [mg/L]	22%	34%	22%	49%	34%	42%
	1> & <4 [mg/L]	58%	55%	60%	47%	56%	53%
	<1 [mg/L]	19%	12%	18%	5%	10%	6%
30-39.9	Dose [mg]	600	400		200		100
	C12h [mg/L]	1.80 (0.43-7.11)	2.27 (0.57-8.42)	1.76 (0.45-6.75)	3.30 (0.87-12.59)	2.32 (0.61-8.87)	3.06 (0.77-12.74)
	>4 [mg/L]	16%	24%	16%	40%	26%	36%
	1> & <4 [mg/L]	60%	60%	60%	53%	59%	55%
	<1 [mg/L]	24%	16%	25%	7%	15%	9%

Note: Data presented as median (5^{th}-95^{th} percentile) or percentage. EM (extensive metabolisers) - 516GG|983TT; IM (intermediate metabolisers) - 516GG|983TC or 516GT|983TT, SM (slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (ultra-slow metabolisers) - 516GG|983CC.

4.10.3 NONMEM control stream

```
; Model descr: FINAL EFV model

$SIZES     LVR=50
$PROBLEM    EFV PK PGx
;-----------------------------------------------------------------------------------------------------------------------------
$INPUT      ID EVENT=DROP SPK STUDY IND WHAT=DROP DAT1=DROP TIME OCC
            OCC_CL OBS AMT DV MDV EVID NRTI TB SITE AGE SEX WT HT WAZ
            HAZ DOSE TRAD_MED TRAD_MED_CODE G516T T983C rs480349 A785G
            rs35599367 rs776746 rs3003596 rs2307424 rs2472677 PGX3 MET
            wghtband FLAG COMMENTS=DROP
;---------------------------------- -----------------------------------------------------------------------------------------
$DATA      EFV_NONMEM_BOTH_14Apr2015v2.csv IGNORE=@ IGNORE=(FLAG.EQ.1)

;---------------------------------- -----------------------------------------------------------------------------------------
$ABBREVIATED COMRES=2
$SUBROUTINE ADVAN13 TRANS1 TOL=9
;---------------------------------- -----------------------------------------------------------------------------------------
$MODEL      NCOMP=3 COMP=(DEPOT DEFDOSE) COMP=(CENTRAL DEFOBS)
          COMP=(PERI)
;-----------------------------------------------------------------------------------------------------------------------------

;; Initial estimates Theta and Omega
$THETA (0,6.853900,15)       ; 1 516GG 983TT TVCL  [L/h]
$THETA (0,62.42090,200)      ; 2 TVV2 [L]
$THETA (0,0.729018,5)         ; 3 TVKA [1/h]
$THETA (0,0.098377,5)         ; 4 ADD error
$THETA (0,0.067533,1)         ; 5 PROP error []
$THETA (0,17.35440,30)        ; 6 TVQ [L/h]
$THETA (0,92.86670,400)       ; 7 TVV3 [L]
$THETA (0,0.803833,8)         ; 8 TVMTT [h]
$THETA (0.1,3.213450,8)       ; 9 LOG NN[]
$THETA (0,2.009010,5)         ; 10 SPK ERROR []
$THETA (0,3.931,10)           ; 11 516GG 983TC TVCL  [L/h]
$THETA (0,0.738663,10)        ; 12 516GG 983CC TVCL  [L/h]
$THETA (0,4.81844,10)         ; 13 516GT 983TT  TVCL  [L/h]
$THETA (0,1.35335,10)         ; 14 516GT 983TC TVCL  [L/h]
$THETA (0,1.94779,10)         ; 15 516TT 983TT TVCL  [L/h]
$THETA (-0.99,0.67467,3)      ; 16 KA ARROW [% change]
$THETA (-0.99,0.578425,3)     ; 17 MTT ARROW [% change]

$OMEGA  0.156951         ; 1 BSVCL
$OMEGA  0  FIX           ; 2 BSVV2
$OMEGA  0  FIX           ; 3 BSVKA
$OMEGA  0  FIX           ; 4 BSVQ
$OMEGA  0  FIX           ; 5 BSVMTT
$OMEGA  0.174540         ; 6 BSVBI0
$OMEGA  0  FIX           ; 7 BSVV3
$OMEGA  BLOCK(1)
 0.229487                ; BOVBIO OCC 1
```

```
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1)
 0.763071                   ; BOVMTT OCC 1
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$OMEGA  BLOCK(1) SAME
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$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1)
 0.080154                   ; BOVCL OCC 1
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
;-------------------------------------------------------------------------------------------------------------------------
$PK
;----- Allometric Scaling ----------------------------------------------------------------------------------------------
  TVWT = 15.4              ; Median weight in kg
  ALLMCL=(WT/TVWT)**0.75
  ALLMV=WT/TVWT

;----- DEFINE VARIABILITY  ---------------------------------------------------------------------------------------------
;----- BSV -------------------------------------------------------------------------------------------------------------
  BSVCL    = ETA(1)
  BSVV2    = ETA(2)
  BSVKA    = ETA(3)
  BSVQ     = ETA(4)
  BSVMTT = ETA(5)
  BSVBIO   = ETA(6)
```

```
  BSVV3    = ETA(7)

;----- BOV ---------------------------------------------------------------------------------------------------------------------
  BOVBIO =ETA(8)                          ;OCC=1 lag doses and pre-dose are treated as same occasion
  IF (OCC.GT.1.5.AND.OCC.LE.2.5) BOVBIO = ETA(9)  ;OCC=2
  IF (OCC.GT.2.5.AND.OCC.LE.3.5) BOVBIO = ETA(10) ;OCC=3
  IF (OCC.GT.3.5.AND.OCC.LE.4.5) BOVBIO = ETA(11) ;OCC=4
  IF (OCC.GT.4.5.AND.OCC.LE.5.5) BOVBIO = ETA(12) ;OCC=5
  IF (OCC.GT.5.5.AND.OCC.LE.6.5) BOVBIO = ETA(13) ;OCC=6
  IF (OCC.GT.6.5.AND.OCC.LE.7.5) BOVBIO = ETA(14) ;OCC=7
  IF (OCC.GT.7.5.AND.OCC.LE.8.5) BOVBIO = ETA(15) ;OCC=8
  IF (OCC.GT.8.5.AND.OCC.LE.9.5) BOVBIO = ETA(16) ;OCC=9

  BOVMTT = ETA(17)                                ;OCC=1
  IF (OCC.GT.1.5.AND.OCC.LE.2.5) BOVMTT = ETA(18) ;OCC=2
  IF (OCC.GT.2.5.AND.OCC.LE.3.5) BOVMTT = ETA(19) ;OCC=3
  IF (OCC.GT.3.5.AND.OCC.LE.4.5) BOVMTT = ETA(20) ;OCC=4
  IF (OCC.GT.4.5.AND.OCC.LE.5.5) BOVMTT = ETA(21) ;OCC=5
  IF (OCC.GT.5.5.AND.OCC.LE.6.5) BOVMTT = ETA(22) ;OCC=6
  IF (OCC.GT.6.5.AND.OCC.LE.7.5) BOVMTT = ETA(23) ;OCC=7
  IF (OCC.GT.7.5.AND.OCC.LE.8.5) BOVMTT = ETA(24) ;OCC=8
  IF (OCC.GT.8.5.AND.OCC.LE.9.5) BOVMTT = ETA(25) ;OCC=9

  BOVKA = ETA(26)                                 ;OCC=1
  IF (OCC.GT.1.5.AND.OCC.LE.2.5) BOVKA = ETA(27) ;OCC=2
  IF (OCC.GT.2.5.AND.OCC.LE.3.5) BOVKA = ETA(28) ;OCC=3
  IF (OCC.GT.3.5.AND.OCC.LE.4.5) BOVKA = ETA(29) ;OCC=4
  IF (OCC.GT.4.5.AND.OCC.LE.5.5) BOVKA = ETA(30) ;OCC=5
  IF (OCC.GT.5.5.AND.OCC.LE.6.5) BOVKA = ETA(31) ;OCC=6
  IF (OCC.GT.6.5.AND.OCC.LE.7.5) BOVKA = ETA(32) ;OCC=7
  IF (OCC.GT.7.5.AND.OCC.LE.8.5) BOVKA = ETA(33) ;OCC=8
  IF (OCC.GT.8.5.AND.OCC.LE.9.5) BOVKA = ETA(34) ;OCC=9

  BOVCL = ETA(35)                                   ;OCC=1
  IF (OCC_CL.GT.1.5.AND.OCC_CL.LE.2.5) BOVCL = ETA(36) ;OCC=2
  IF (OCC_CL.GT.2.5.AND.OCC_CL.LE.3.5) BOVCL = ETA(37) ;OCC=3
  IF (OCC_CL.GT.3.5.AND.OCC_CL.LE.4.5) BOVCL = ETA(38) ;OCC=4
  IF (OCC_CL.GT.4.5.AND.OCC_CL.LE.5.5) BOVCL = ETA(39) ;OCC=5
  IF (OCC_CL.GT.5.5.AND.OCC_CL.LE.6.5) BOVCL = ETA(40) ;OCC=6
  IF (OCC_CL.GT.6.5.AND.OCC_CL.LE.7.5) BOVCL = ETA(41) ;OCC=7


;----- DEFINE POPULATION PARAMETERS ----------------------------------------------------------------------------------------
;----  CL BASED ON MIXTURE MODEL ------------------------------------------------------------------------------------------
EST=MIXEST

PGX_EST=PGX3                                        ; patients with available genotype

IF (PGX_EST.EQ.99.AND.MIXNUM.EQ.1)  PGX_EST=1     ; 516GG 983TT - from mixture model
IF (PGX _EST.EQ.99.AND.MIXNUM.EQ.2) PGX_EST=2     ; 516GG 983TC - from mixture model
IF (PGX _EST.EQ.99.AND.MIXNUM.EQ.3) PGX_EST=3     ; 516GG 983CC - from mixture model
```

```
IF (PGX _EST.EQ.99.AND.MIXNUM.EQ.4) PGX_EST=4     ; 516GT 983TT- from mixture model
IF (PGX _EST.EQ.99.AND.MIXNUM.EQ.5) PGX_EST=5     ; 516GT 983TC - from mixture model
IF (PGX _EST.EQ.99.AND.MIXNUM.EQ.6) PGX_EST=6     ; 516TT 983TT- from mixture model

IF (PGX _EST.EQ.1) THEN         CLPGX = THETA(1)     ; 516GG 983TT
ELSEIF (PGX _EST.EQ.2) THEN   CLPGX = THETA(11)   ; 516GG 983TC
ELSEIF (PGX _EST.EQ.3) THEN   CLPGX = THETA(12)   ; 516GG 983CC
ELSEIF (PGX _EST.EQ.4) THEN   CLPGX = THETA(13)   ; 516GT 983TT
ELSEIF (PGX _EST.EQ.5) THEN   CLPGX = THETA(14)   ; 516GT 983TC
ELSEIF (PGX _EST.EQ.6) THEN   CLPGX = THETA(15)   ; 516TT 983TT
   ENDIF
;-------------------------------------------------------------------------------------------------------------------------------

  TVCL   = CLPGX * ALLMCL          ; typical value of CL
  TVV2  = THETA(2) *ALLMV         ; typical value of V
  TVQ    = THETA(6) *ALLMCL       ; typical value for Q
  TVV3  = THETA(7) *ALLMV         ; typical value for V3
  TVNN = EXP(THETA(9))             ; typical number of transit compartments
  TVBIO= 1

  COVKA=0
  IF(STUDY.EQ.1) COVKA=THETA(16)    ; % increase in ka for ARROW
  TVKA = THETA(3) *(1+COVKA)          ; typical value of KA

  COVMTT=0
  IF(STUDY.EQ.1) COVMTT=THETA(17) ; % increase in MTT for ARROW
  TVMTT= THETA(8)*(1+COVMTT)       ; typical mean transit time


;----- DEFINE INDIVIDUAL PARAMETERS ----------------------------------------------------------------------------
 CL     = TVCL *EXP(BSVCL+BOVCL)           ; individual value of CL
 V2    = TVV2 *EXP(BSVV2)                    ; individual value of V2
 KA    = TVKA *EXP(BSVKA+BOVKA)          ; individual value of KA
 Q     = TVQ   *EXP(BSVQ)                     ; individual value of Q
 V3    = TVV3  *EXP(BSVV3)                   ; individual value of V3
 MTT = TVMTT*EXP(BSVMTT+BOVMTT)    ; individual value of MTT
 NN   = TVNN                                    ; individual value of NN
 BIO   = TVBIO *EXP(BSVBIO+BOVBIO)       ; individual value of BIO

;------------------ TRANSFER RATE CONSTANTS ---------------------------------------------------------------------------
 K    = CL/V2
 K23  = Q/V2
 K32  = Q/V3

 ; RESET code for Cmax Tmax
IF (NEWIND/=2.OR.EVID>=3) THEN
          COM(1)=0
          COM(2)=0
          TIMEDOSE = TIME
          AMOUNTDOSE = AMT
ENDIF
```

```
;---------------------  TRANSIT  ----------------------------------------------------------------------------------------

F1=0                        ; I need to set bioavailability in compartment 1 to 0

KTR = (NN+1)/MTT

IF (NEWIND/=2.OR.EVID>=3) THEN ; new individual, or reset event
   ; The values read here will be stored in TDOS and PD in this very PK call
        TNXD=TIME ; Time of the dose
        PNXD=AMT ; Amount. If it's zero, the DE is deactivated.
ENDIF

TDOS=TNXD ; This will either save here the temporary values if it's a new individual...
PD=PNXD ; ...or the values which were read one record ahead during the execution of the previous record.

IF(AMT.GT.0) THEN ; This reads one record ahead and stores the data to be used when running the following record
        TNXD=TIME
        PNXD=AMT
ENDIF

LNGAM = NN*LOG(NN)-NN+LOG(NN*(1+4*NN*(1+2*NN)))/6+0.572364942
; approximation of log of gamma(n), 0.572364942 is LOG(PI)/2
; To speed up the computation, I calculate here all the non-time-varying quantities used in $DES
PIZZA=LOG(BIO*PD*KTR+0.00001)-LNGAM  ;  without  +0.00001,  it  won't  work  with  ETAs  in bioavailability

;----------------- Initialisation for DES solver  ----------------------------------------------------------------------
; SS option causes model to crash due to some low concentrations
; Instead initialise the compartments with approximated SS Cmin (see below)
; Based on individual parameter values
; 2 CMT model - CALCULATE ALPHA AND BETA

 K20=K
 TMP0 = K20*K32                          ; ALPHA * BETA
 TMP1 = K20+K23+K32                      ; ALPHA + BETA
 TMPDELTA = SQRT(TMP1*TMP1 - 4* TMP0) ; SQ ROOT FROM EQUATION
 SOL1 = (TMP1 + TMPDELTA) / 2            ; ALPHA
 SOL2 = (TMP1 - TMPDELTA) / 2            ; BETA

 ALFA  = SOL1
 BBETA = SOL2
 VBETA = CL / SOL2                        ; VBETA=CL/BETA – approx of volume in terminal phase

; SEQUENCE FOR INITIATION OF $DES
 TAU_EQ=MTT+1/KA
 KA_EQ=1/TAU_EQ                          ; because we’re using TRANSIT absoprtion

 ; treat both compartments as one single compartment at steady state
 ; Cmin cent = approx Cmin per (should be approx at equilibrium)
```

```
; SUBSTITUTE V BY VBETA AND K BY SOL2 (BETA) – just an approximation
; Followed with additional 5 days of dosing to make sure patient is at steady state

 CMIN = ( (BIO*DOSE * KA_EQ) /(VBETA*(KA_EQ - SOL2)))*( (1 / (1- EXP(-SOL2 * 24)))-(1 / (1- EXP(-
KA_EQ*24))) )

 A_0(1)= 0.0001                ; initialise absorption CMT
 A_0(2)= V2 * CMIN             ; initialise central CMT
 A_0(3)= V3 * CMIN             ; initialise peri CMT

$DES
TEMPO=T-TDOS                   ; this is time after dose, it should always be >= 0
KTT=0

DADT(1)=0
;incorporated code to switch off depot compartment after 16hr to speed up model runs
IF(PD.GT.0.AND.TEMPO.GT.0.AND.TEMPO.LT.16) THEN ; This happens only id PD>0, so only if a dose
has been detected
            KTT=KTR*(TEMPO)
            DADT(1)=EXP(PIZZA+NN*LOG(KTT)-KTT)-KA*A(1)
            DADT(2)= KA*A(1)- K23*A(2) + K32*A(3) - K*A(2)
          ELSE
            DADT(1)=0
            DADT(2)= - K23*A(2) + K32*A(3) - K*A(2)
ENDIF

  DADT(3)= K23*A(2) - K32*A(3)

; For Cmax Tmax
CP = A(2)/V2                          ; plasma concentration
TIMEAFTERDOSE=T-TIMEDOSE
 IF (CP.GE.COM(1)) THEN
         COM(1) = CP                  ; CMAX
         COM(2) = TIMEAFTERDOSE     ; time of CMAX
ENDIF

;------------------------------------ MIXTURE MODEL  ---------------------------------------------------------------------
$MIX      NSPOP=6
; proportions set to ones observed in patients
P(1) = 0.33     ; prop of 516GG 983TT
P(2) = 0.06     ; prop of 516GG 983TC
P(3) = 0.01     ; prop of 516GG 983CC
P(4) = 0.34      ; prop of 516GT 983TT
P(5) = 0.07     ; prop of 516GT 983TC
P(6) = 0.19      ; prop of 516TT 983TT
;-----------------------------------------------------------------------------------------------------------------------------
$ERROR
 IPRED = A(2)/V2                ; Individual prediction PK (CMT=2)
 IRES    = DV - IPRED           ; Individual residual

 WA    = THETA(4)               ; Additive error
```

```
 WP    = IPRED * THETA(5)              ; Prop error
 W     = SQRT(WA**2 + WP**2)           ; Weighting factor for residuals
 IF(W.LE.0.0001) W = 0.0001            ; Protection against division with 0

 IF(SPK.GT.0.5.AND.SPK.LT.1.5) W = W*(THETA(10))     ; Additional error term for sparse data

 IWRES = IRES / W
 Y     = IPRED + W*EPS(1)     ; Model prediction of observed PK value with additive + proportional error

AA1 = A(1)                    ; abs CMT
AA2 = A(2)                    ; central CMT
AA3 = A(3)                    ; peri CMT
CMAX = COM(1)                 ; CMAX
TMAX = COM(2)                 ; TIME OF CMAX
AUC_INF =  DOSE*BIO/CL  ; because no temporal effects
VAR_AUC = (BSVBIO + BOVBIO) - (BSVCL + BOVCL)

TVPC=TIME-120                 ; approx for plotting VPC/ (time - 5x 24h ->imputed 5 days of dosing)
TAD =TIME-TIMEDOSE

IF(AMT.GT.0) THEN
          TIMEDOSE = TIME
          AMOUNTDOSE = AMT
; Reset CMAX code when a new dose is given
          COM(1)=0
          COM(2)=0
ENDIF

 IF (ICALL==4.AND.Y.LE.0.1) Y=0.05 ; prevents negative simulated values for VPC
;-----------------------------------------------------------------------------------------------------------------------------
$SIGMA  1  FIX  ; Scaled RUV variance - all error is going to the thetas and it makes SD
$ESTIMATION MSFO=msf910 MAXEVAL=0 PRINT=1 METHOD=1 INTER MCETA=1000
        RANMETHOD=4P ETASTYPE=1 NONINFETA=1 NOABORT NSIG=3 SIGL=9
        ATOL=9
$ESTIMATION MSFO=msf910 MAXEVAL=9999 PRINT=1 METHOD=1 INTER MCETA=5
        RANMETHOD=4P ETASTYPE=1 NONINFETA=1 NOABORT NSIG=3 SIGL=9
        ATOL=9

$COVARIANCE PRINT=E SIGL=12 TOL=10 ATOL=12; values for high precision

$TABLE     WRESCHOL ID DV OCC OCC_CL EVID MDV TAD TVPC TIME PRED
        IPRED DOSE AA1 AA2 AA3 IWRES WRES CWRES NPDE OBJI
        ESAMPLE=100 NOPRINT ONEHEADER FILE=sdtab910
$TABLE     ID CL V2 KA BIO K Q V3 AUC_INF VAR_AUC NN MTT BSVCL BSVV2
        BSVKA BSVQ BSVV3 BSVBIO BSVMTT BOVBIO BOVKA BOVMTT WA WP
        NOPRINT NOAPPEND ONEHEADER FILE=patab910
$TABLE     ID AGE HT WT WAZ HAZ NOPRINT NOAPPEND ONEHEADER
        FILE=cotab910
$TABLE     ID SPK STUDY IND SEX SITE DOSE NRTI TB TRAD_MED
        TRAD_MED_CODE PGX3 wghtband G516T T983C rs480349 A785G
        rs35599367 rs776746 rs3003596 rs2307424 rs2472677 MET PGX_EST
```

```
        NOPRINT NOAPPEND ONEHEADER FILE=catab910
$TABLE      ID DV CP OCC SPK STUDY IND FLAG OCC_CL EVID MDV TAD TVPC
        TIME AA1 AA2 AA3 CL V2 KA BIO K Q V3 NN MTT BSVCL BSVV2
        BSVKA BSVQ BSVV3 BSVBIO BSVMTT BOVBIO BOVKA BOVMTT AUC_INF
        VAR_AUC TMAX CMAX CMIN WA WP AGE HT WT WAZ HAZ HT WT WAZ
        HAZ SEX SITE DOSE NRTI TB TRAD_MED TRAD_MED_CODE PGX3 PGX_EST MET
        wghtband G516T T983C rs480349 A785G rs35599367 rs776746
        rs3003596 rs2307424 rs2472677 NOPRINT NOAPPEND ONEHEADER
        FILE=mytab910.csv FORMAT=,
; Xpose can read these tables

; there must be one empty line after the last command line

;--------------------------------
```

CHAPTER 5: PLASMA EFAVIRENZ EXPOSURE, SEX, AND AGE PREDICT VIROLOGICAL RESPONSE IN HIV-INFECTED AFRICAN CHILDREN.

5.1 Abstract:

Background: Due to insufficient evidence in children, target plasma concentrations of efavirenz are based on studies in adults. Our analysis aimed to evaluate the paediatric therapeutic thresholds and characterise the determinants of virological suppression in African children.

Methods: We analysed data from 128 African children (aged 1.7-13.5 years) treated with efavirenz, lamivudine, and either abacavir, stavudine, or zidovudine, and followed up to 36 months. Individual pharmacokinetic measures (C12h, C24h and AUC_{0-24}) were estimated using population-pharmacokinetic modelling. Cox multiple failure regression and multivariable fractional polynomials were used to investigate the risks of unsuppressed viral load associated with efavirenz exposure and other factors amongst 106 initially treatment-naïve children, and likelihood profiling used to identify the most predictive pharmacokinetic thresholds.

Results: The risk of viral load >100 copies/mL decreased by 42% for every 2-fold increase in efavirenz mid-dose concentration (95% CI: 23-57%; p<0.001). The most predictive PK thresholds for increased risk of unsuppressed viral load were: C12h 1.12 mg/L (HR=6.14; 95% CI: 2.64-14.27), C24h 0.65 mg/L (HR=6.57; 95% CI: 2.86-15.10), and AUC_{0-24} 28 mg·h/L (HR=5.77; 95% CI: 2.28-14.58). Children over 8 years old had a more than 10-fold increased risk of virological non-suppression (p=0.005); among children under 8 years old boys had a 5.31 times higher risk than girls (p=0.007). Central nervous system AEs were infrequently reported.

Conclusions: Our analysis suggests the minimum target C24h and AUC_{0-24} could be lowered in children. Our findings should be confirmed in a prospective paediatric trial.

5.2 Introduction

The non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz is recommended by the World Health Organization (WHO) as part of first-line treatment for HIV-infected children aged over 3 years.[11] Due to its high potency, long half-life, and availability of low cost generic formulations, efavirenz continues to be one of the most widely used antiretrovirals in Africa and worldwide.[155] The mid-dose plasma concentration target of 1.0-4.0mg/L derived from adult clinical monitoring data is customarily also applied to trough concentrations.[93,94] In adults, systemic exposure below that range is associated with virological failure and higher exposures with central nervous system (CNS) toxicities.[69,94,168] The same target range is used in children, however rigorous analyses have not confirmed the optimal therapeutic range for this age group.[32,99,100,105,284]

The main objective of a pharmacokinetic/pharmacodynamic (PK/PD) analysis is to quantify the relationships between drug dose, exposure and response, identifying factors affecting drug disposition and efficacy.[93] While the high variability in efavirenz PK in children has been thoroughly studied,[34,74,75,162] analyses successfully relating observed drug exposures to treatment response and detecting other determinants of treatment failure are limited.[32,100,101] Factors affecting efavirenz effectiveness have often been investigated independently of drug concentrations with inconclusive findings across studies, [99,100,149–151,279] similarly the effect of high efavirenz exposure on increased risk of CNS AEs is unconfirmed in children.[96–99]

The recent results of ENCORE1,[102,103] showing that the standard 600mg efavirenz dose can be reduced to 400mg daily without loss of efficacy in non-pregnant adults, have prompted discussions on the validity of the widely accepted efficacy thresholds of >1 mg/L, for a mid-dose interval or trough concentration,[103] and suggest that the target range used for children should also be re-evaluated. Our analysis therefore aimed to characterise associations between systemic exposure to efavirenz and risk of virological non-suppression and CNS AEs over the longer-term, to identify factors affecting virological non-suppression independently of systemic exposure and to validate the lower boundary of the therapeutic range for efavirenz in African children.

5.3 Methods

5.3.1 Population and Study Design

As described previously,[382] the CHAPAS-3 study enrolled HIV-infected antiretroviral therapy (ART) naïve and experienced children 13 years or younger in four sites in Uganda and Zambia. Of 478 participants, 128 received efavirenz and lamivudine combined with either abacavir, stavudine or

zidovudine. Children switched to boosted protease inhibitor-based second-line ART for clinical or immunological failure following WHO 2010 guidelines. Samples for PK analysis were taken at week 6, week 36, and every 24 weeks thereafter. Efavirenz pharmacokinetics were described previously.[415] Viral load (VL) was measured retrospectively in stored plasma samples taken at enrolment and weeks 48, 96, and 144; and at weeks 36, 60, 84, 108, and 132 when PK samples were taken. An undetectable VL was defined as <100 copies/mL, the lower limit of detection as many samples had to be diluted due to low volumes.

5.3.2 Statistical analysis

Empirical Bayesian Estimates for the individual parameters from the previously developed population PK (POP-PK) model were used to estimate steady-state mid-dose efavirenz concentrations (C12h, defined as plasma concentration 12 h after dose), trough concentrations (C24h, 24 h after dose), and $AUC_{0\text{-}24}$ (area under the curve) for each child at each included timepoint.[415]

Children followed for <48 weeks were excluded from all analyses. For a preliminary analysis, VL response was categorized as: suppressed (<100 copies/ml achieved within 48 weeks of treatment initiation and maintained throughout the study), single rebound (<100 copies/ml within 48 weeks and a single viral rebound >100 copies/ml), multiple rebounds (<100 copies/ml within 48 weeks and multiple viral rebounds) and never suppressed <100 copies/mL. Treatment-experienced children who were virologically suppressed at study enrolment were analysed separately. Since multiple PK exposures were available for each child, the geometric-mean exposure value (derived from all PK visits) for each child were compared between groups using Kruskal-Wallis and ranksum tests. Categorical factors were compared between groups using Fisher's exact test.

The effects of PK on virological non-suppression (>100 copies/ml) were then estimated using Cox proportional hazards regression models (Andersen-Gill repeated outcomes framework) with Efron approximation in R (survival package),[380,402–404] including only VLs measured on PK sampling days from week 36 onwards in children treatment-naïve at enrolment. Samples taken before initial viral suppression were excluded, unless children never suppressed during the study. Each time interval ran from the preceding to current VL (classified as suppressed vs non-suppressed "event"), and the estimated PK parameters at the current VL were applied to the whole time interval. Non-linearity in effect of PK exposures was explored visually using smoothed splines, and tested using fractional polynomials (using Stata 14.0 mfp).[401] The best-fitting (lowest Akaike Information Criterion [AIC]) dichotomous threshold was identified by profile likelihood. Because PK parameters were estimated and not observed, we used a re-simulation approach to assess the impact of unobserved variability on selection of the dichotomised threshold. The original data set was re-simulated 500 times introducing

a normally distributed random error on each of the exposure parameters, set to the unexplained residual variability from the POP-PK model (additive error 0.101 mg/L, proportional error 0.0672).[415] The results were used to derive 95% confidence interval (CI) for the threshold (2.5th and 97.5th percentile of distribution of most predictive cut-offs from 500 runs).

For each PK exposure threshold identified in this study and the previously proposed efficacy thresholds, we calculated sensitivity (proportion of samples correctly predicted as not-suppressed), specificity (proportion of samples correctly predicted as suppressed), accuracy (overall proportion of correctly predicted samples), positive predictive value (proportion of samples with exposure below the threshold not suppressed), and negative predictive value (proportion of samples with exposure above the threshold that were suppressed).

Finally, we used backwards elimination (exit p=0.05, retaining all levels of categorical factors where any were p<0.05) to consider the additional independent effects of covariates on non-suppression with associations (p<0.2) in univariable models. Categorical covariates included nucleoside reverse transcriptase inhibitor (NRTI) backbone (abacavir, zidovudine, stavudine), sex, clinical site, mother as primary carer, self-reported missing doses in previous 4 weeks. Continuous variables included pre-ART viral load, CD4% pre-ART and at time of PK/VL measurement, age, weight-for-age Z-score (WAZ),[416] height-for-age Z-score (HAZ)[416] and MEMS-adherence (proportion of days without drug intake based on MEMS-cap container openings in the interval between previous and current measurement [truncated at a lower limit of 0.5]. The only covariate with incomplete information was adherence; where no data was available for current interval the previous MEMS-adherence was carried forward. If no MEMS-adherence data were available for the child (N=19) we imputed the median of all treatment-naïve patients). Only one child had concurrent co-administration of anti-TB drugs, so this factor was not considered. Non-linear effects in continuous variables were included using fractional polynomials (Stata mfp). Interactions between factors included in the final model were investigated and included if p<0.05. How much of the PK exposure effect could be explained by metabolic status based on 516GT|983TC single nucleotide polymorphisms (SNP)[71] was then investigated by adding this factor into the final model.

5.3.3 CNS adverse events

Specific CNS toxicities relating to cognitive or motoric functions were solicited at every follow up visit (concentration, vivid dreams/nightmares, sleepiness/sleepwalking, waking at night, difficulty waking in the morning, dizziness) and graded between 1-3 (mild-severe). Incidence of CNS AEs was compared between groups using Fisher's exact test.

Table 5.1 Demographic characteristics and model-derived PK parameters in different suppression groups of children in CHAPAS-3 treated with efavirenz

		Treatment-naïve at enrolment					Treatment – experienced at enrolment	
		Suppressed	Single Rebound	Multiple Rebound	Never Suppressed	p*		
Number of children		73	19	10	7		14	p**
Baseline	Age [years]	4.3 (3.5-4.7)	3.9 (3.6-4.5)	3.5 (3.3-3.8)	3.5 (3.2-8.5)	0.208	7.3 (5.7-8.5)	<0.001
	Weight [kg]	14.0 (12.4-16.0)	14.5 (13.5-16.0)	13.4 (12.8-15.7)	12.3 (12.0-17.5)	0.513	20.1 (19.3-22.6)	<0.001
	CD4% [%]	18.5 (11.0-24.0)	17.2 (7.3-22.1)	19.5 (15.5-24.5)	19.5 (10.3-19.5)	0.565	35.6 (31.4-37.8)	<0.001
	CD4 [cells/mL]	707 (497-977)	648 (204-904)	684 (477-1047)	618 (201-913)	0.429	915 (807-1249)	<0.001
	Viral Load [copies/mL]	172165 (63250-338685)	93160 (23415-218830)	175390 (130080-606275)	149080 (69065-354885)	0.268	< 100	<0.001
	Sex (M/F)	29/44	10/9	8/2	4/3	0.133	9/5	0.091
Metabolic Subgroup†	FM	24	7	1	2	0.073	5	0.173
	IM	28	6	8	3		6	
	SM	21	6	--	2		3	
	USM	--	--	1	--		--	
NRTI	Stavudine	23	7	5	1	0.738	5	0.908
	Zidovudine	26	6	2	4		4	
	Abacavir	24	6	3	2		5	
PK measure	AUC_{0-24} [mg·h/L]	57.1 (37.1-101.4)	57.8 (45.9-121.8)	46.6 (42.8-78.0)	36.8 (13.6-74.0)	0.360	77.7 (58.5-114.2)	0.142
	C12h [mg/L]	2.25 (1.43-4.11)	2.22 (1.77-4.96)	1.93 (1.65-3.08)	1.40 (0.5-2.83)	0.367	3.12 (2.38-4.43)	0.155
	C24h [mg/L]	1.54 (0.95-3.15)	1.43 (1.19-3.69)	1.24 (1.01-1.77)	0.76 (0.31-1.82)	0.223	2.27 (1.630-3.53)	0.123
	Cmax [mg/L]	4.20 (3.03-6.28)	4.42 (3.22-6.59)	3.81 (3.24-6.99)	3.50 (1.07-5.62)	0.606	5.33 (4.70-6.66)	0.159
	CL [L/h]	5.6 (3.2-7.7)	5.6 (2.4-7.3)	6.2 (5.2-7.6)	9.1 (5.9-9.2)	0.382	6.5 (4.3-8.3)	0.479
Adherence (MEMScaps)‡		1.00 (0.97-1.00)	0.98 (0.95-1.00)	0.98 (0.91-0.99)	0.87 (0.69-0.95)	0.010	0.97 (0.91-1.00)	<0.001

Note: Presented values are number or median (IQR)

*Kruskal Wallis or Fisher's Exact test comparing 4 groups of originally treatment-naïve children only. **Kruskal Wallis or Fisher's Exact test comparing 5 groups including children who were treatment-experienced at enrolment. †EM (extensive metabolisers) - 516GG|983TT; IM (intermediate metabolisers) - 516GG|983TC or 516GT|983TT, SM (slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (ultra-slow metabolisers) - 516GG|983CC; ‡Data from 104 patients (91 treatment-naïve and 13 treatment-experience at enrolment)

5.4 Results

In total 128 children (14 treatment-experienced) received efavirenz in CHAPAS-3 and contributed a total of 1482 PK measurements from 570 PK visits, 345 with paired VL measurements. Five children with <48 week follow-up were excluded from all analyses and a further 3 children with no paired PK-VL measurements were excluded from the Cox model. Table 5.1 shows child characteristics and model-derived PK parameters in each suppression group. 67% of children (n=73) who were treatment-naïve at enrolment achieved and maintained viral suppression <100 copies/ml, 17% (n=19) had a single episode of viral rebound while 15% (n=17) had multiple viral rebounds or never suppressed. There were no statistically significant differences in baseline (pre-ART) demographic characteristics or geometric-mean PK parameters across study follow-up between these four groups. However, there was a trend to lower average exposures and higher average clearance among treatment-naïve children who never suppressed, compared to children who achieved and sustained viral suppression (pairwise ranksum: C12h p=0.11, C24h p=0.07, AUC p=0.12, CL p=0.08). Average adherence was also significantly lower in those who never suppressed (p=0.004 vs sustained suppression), whereas there was no difference in demographics between these two groups.

Of 14 children who were treatment-experienced (and virologically suppressed) at enrolment, one had a single episode of viral rebound, the rest remained suppressed throughout follow-up. Children treatment-experienced at enrolment differed significantly from the treatment-naïve children in baseline characteristics, and had higher geometric-mean efavirenz exposures (all p<0.05 vs naïve children combined) but no difference in average clearance (p=0.63). Average adherence was marginally lower in treatment-experienced compared to the naïve patients (0.97 vs 1.00, p=0.006).

5.4.1 Hazard of virological non-suppression

Repeated measures Cox proportional hazards regression models fitted to 345 matched PK-VL samples from 106 treatment-naïve children indicated that the risk of virological non-suppression increased approximately uniformly with each fold-change in PK exposures (i.e. a log transform of PK exposure) (Table 5.2).

Table 5.2 Univariable Cox proportional hazards regression models for efavirenz C12h, C24h and AUC

PK Par		Change in risk per unit increase in absolute exposure	Change in risk per doubling of exposure (per unit increase in log transformed exposure)	Change in risk change at threshold for dichotomized exposure variables (Supplement 1)
C12h [mg/L]	**HR (95% CI)**	0.87 (0.69-1.10)	0.58 (0.43-0.77)	6.14 (2.64-14.27) (vs C12h>1.12mg/L)
	p-value	0.241	< 0.0001	< 0.0001
	AIC	324.11	305.76	304.55
C24h [mg/L]	**HR (95% CI)**	0.86 (0.67-1.11)	0.60 (0.46-0.78)	6.57 (2.86-15.10) (vs. C24h>0.65mg/L)
	p-value	0.246	< 0.0001	< 0.0001
	AIC	324.67	304.18	302.82
AUC [mg·h/L]	**HR (95% CI)**	0.9941 (0.9843-1.0040)	0.57 (0.42-0.76)	5.77 (2.28-14.58) (vs. AUC>28mg·h/L)
	p-value	0.247	< 0.0001	< 0.0001
	AIC	324.04	305.70	307.74

HR=Hazard ratio.

Note: Lowest AIC values indicates the models best describing the association with viral non-suppression. Log transform was the best fitting fractional polynomial for C24h; for C12h and AUC the best fitting transform was inverse square root. However, the difference in AIC compared to log-transform was very small in both cases (+0.47 and +0.97) and so the log transform is presented above for comparability with C24h and ease of interpretation.

Profile likelihood identified thresholds of 1.12 mg/L (95% CI from re-simulations 0.47-1.56 mg/L) for C12h, 0.65 mg/L (95% CI 0.25-1.27) for C24h and 28 mg·h/L (95% CI 20.47-32.22) for $AUC_{0\text{-}24}$ as the best dichotomised thresholds for predicting virological suppression (Figure 5.1 in the Appendix to Chapter 5). For AUC, the model including log exposure was superior, whereas for C12h and C24h, a dichotomised threshold provided a better model fit, but these margins were relatively small (Table 5.2).

5.4.2 Multivariate analysis

The three PK exposures were highly correlated (spearman rho >0.98), which could be expected since they were derived from the same population-PK model. We therefore only considered C12 in multivariable models. The only other factors associated (p<0.2) with virological non-suppression in univariate analyses were sex, site, current age and current WAZ. However only C12h, sex, site and current age were independent predictors (selected using backwards elimination). There was a significant interaction between sex and age (p=0.01), i.e. age was an effect modifier for sex. To represent this interaction we dichotomised age at 8 years (based on univariate profile likelihood as

Table 5.3 Univariate and multivariate predictors of virological suppression for children in CHAPAS-3 treated with efavirenz

Factor	Univariate		Final Multivariate Model	
	HR (95% CI)	p	HR (95% CI)	p
C12h (per doubling)	0.58 (0.43-0.77)	<0.001	0.61 (0.50-0.76)	<0.001
Sex: male vs female	2.77 (1.01-7.64)	0.048	(see interaction below)	
Current age (ref. <8 years)	5.45 (1.85-16.06)	0.002	(see interaction below)	
Sex and age (ref girl <8y)	Boy <8y: 6.14 (2.01-18.77) Girl >8y: 16.63 (4.05-68.37) Boy >8y: 25.50 (3.37-193.13)	0.001 <0.001 0.002	Boy <8y: 5.31 (1.58-17.82) Girl >8y: 15.82 (2.97-84.27) Boy >8y: 12.47 (1.31-119.08)	0.007 0.001 0.028
Site (ref. S1)	S2: 0.22 (0.06-0.77) S3: 0.39 (0.11-1.38) S4: 2.48 (0.69-8.99)	0.018 0.146 0.166	S2: 0.73 (0.18-2.88) S3: 1.04 (0.23-4.82) S4: 4.96 (1.38-17.79)	0.653 0.956 0.014
WAZ (per unit higher)	0.66 (0.49-0.88)	0.005	-	

Note: as the final multivariable model identified a significant interaction between age and sex, this interaction is also presented unadjusted for other factors in the univariable column. Final model selected using backwards elimination, see methods. Interaction between continuous age and sex (p=0.01) dichotomised at the optimal age threshold for presentation. HR – hazard ratio; CI – confidence interval;

for PK-exposures). Adjusting for other factors, the hazard of virological non-suppression for boys <8 years was six times greater than girls of similar age (Table 5.3). Older children had increased risk of virological non-suppression compared to younger children, but there was no evidence of a difference between boys and girls >8 years (p=0.76). The hazard of virological non-suppression was significantly higher in the smallest site, which contributed only 5 children. There was marginal evidence that poorer MEMS-adherence independently increased the hazard of virological non-suppression (p=0.065; effects of other factors, including C12h, were similar to Table 5.3). The remaining factors, including metabolizer status (p=0.27) did not have an effect on viral non-suppression (p>0.1).

5.4.3 CNS adverse events

Despite being solicited at every follow-up visit, only 18 CNS AEs were reported in 11 children (3 problems with concentration, 4 vivid dreams, 2 sleep walking, 2 difficulties waking up in the mornings, 3 waking up at night, 4 dizziness; all but one graded mild). These 11 children were 5 slow, 4 intermediate and 2 extensive metabolisers (exact p=0.41). Nine children reported one of these AEs <24 weeks after treatment initiation. Only 2 children reported AEs on repeated occasions (both slow metabolisers), of which only 1 had a paired PK-sample: plasma efavirenz 4h after dose was 45 mg/L, but the child was incorrectly receiving 600mg instead of a 400mg dose.

5.5 Discussion

We observed that efavirenz concentrations were related to virological non-suppression in African children in a non-linear manner, a 2-fold increase in efavirenz exposure decreased the risk of virological non-suppression by over 40%. Some previous studies failed to detect a similar association,[99,167,284] which could be due to a number of reasons: their follow-up time was short, they had only a single outcome at one time point, they were underpowered to characterise the PK/PD relationship or tried to simplify it by a linearisation.[96,99,284] To avoid such limitations our study analysed a unique set of matched PK/VL longitudinal data using Cox multiple failure regression, allowing for repeated within-child measurements, similar to Van Leth[234] and Brundage.[101] This approach enabled us to identify the most predictive dichotomous threshold related to increased risk of VL >100 copies/mL for each PK parameter using profile likelihood, allowing for uncertainty in estimated PK exposures by a resampling approach.

Table 5.4 Comparison of previously published treatment targets for efavirenz concentrations and AUC and most predictive thresholds derived in this analysis.

	C12h [mg/L]				C24h [mg/L]				AUC [mg·h/L]					
Threshold	1.0 [94]		1.12		1.0 [94]		0.65		49[100]		60[96]		28	
HR	6.36		6.14		3.96		6.57		3.16		3.84		5.77	
95% CI	2.53-15.96		2.64-14.27		1.73-9.03		2.86-15.10		1.39-7.16		1.56-9.44		2.28-14.58	
AIC	305.35		304.55		315.07		302.82		319.86		318.12		307.74	
Samples not-sup/sup (n)	< T	> T	< T	> T	< T	> T	< T	> T	< T	> T	< T	> T	< T	> T
	17/ 27	21/ 281	19/ 34	19/ 274	21/ 66	17/ 242	19/ 32	19/ 276	24/ 101	14/ 207	30/ 153	8/ 155	18/ 32	20/ 276
Sensitivity	44.7%		50.0%		55.3%		50.0%		63.2%		78.0%		44.7%	
Specificity	91.2%		88.9%		78.5%		89.6%		67.1%		50.2%		90.23%	
Accuracy	86.1%		84.7%		76.0%		85.3%		66.8%		53.5%		85.2%	
Positive Predictive Value	38.6%		35.8 %		24.1%		37.3%		19.2%		16.4%		36.2%	
Negative Predictive Value	93.0%		93.5%		93.4 %		93.5%		95.1 %		95.1%		92.9%	

HR –hazard ratio, 95% CI – 95% confidence interval, AIC – Akaike information criterion, PK – pharmacokinetic, T – cut-off target. In grey – cut-offs proposed by this analysis, in white – previously published cut-offs.

Comparing our findings with previously proposed cut-offs (Table 5.4), the 1.12 mg/L threshold we obtained for C12h does not differ markedly in sensitivity, specificity, or negative predictive power from the 1.0 mg/L value proposed by Marzolini.[94] However, our cut-offs for C24h and $AUC_{0\text{-}24}$ (0.65 mg/L and 28 mg·h/L, respectively) are lower than previously derived targets,[32,94,100,101,234] and substantially improved specificity, accuracy and positive predictive power, while maintaining a negative predictive power comparable with previously suggested therapeutic thresholds. Whilst our revised cut-offs require independent validation in a prospective paediatric trial, they were determined from the PK/PD relationship rather than using arbitrary percentiles of PK-exposure distribution.

The results of the ENCORE1 study question the validity of a 1 mg/L efficacy threshold in adults[103] but due to low failure rates in the study the authors failed to detect a significant relationship between efavirenz exposure and the virological outcome.[167] Due to design, analytical and population differences, our study was able to define efavirenz exposure thresholds associated with increased risks of virological non-suppression. Our findings should not be extrapolated to adults. Efavirenz clearance in children is relatively higher than in adults, which could affect the suggested cut-offs, especially for C24h and AUC. Furthermore, other differences in PK or pathophysiology between those populations

Table 5.5 Comparison of efavirenz exposure targets and predictors of virological outcome in paediatric studies

Reference	Derived PK Targets	Predictors of virologic failure		n	Method	VL Target [copies/mL]
		PK	Covariates			
Starr *et al.*[96]†	AUC = 60 – 120 mg·h/mL	Not analysed	**Uni:** (A)* $\log_2$ bCD4% , WAZ, bVL / (B)* WAZ, bVL **Multi:** (A)* WAZ, bVL / (B)* bVL	57	Cox	400 (A)* 50 (B)*
Brundage *et al.*[101]	AUC > 59 mg·h/mL	AUC	**Uni:** IPAM, bVL, bCD4%, WAZ **Multi:** IPAM, bVL, AUC	50	Cox, TSSA	400
Hirt *et al*.[32]	C_{min} > 1.1 mg/L AUC > 51 mg·h/L	C_{min}, AUC	Not analysed	48	Fisher's exact test	300
Fletcher *et al*.[100]	AUC > 49 mg·h/mL	AUC	Not analysed	50	logistic regression	400
Janssens *et al*.[279]	Not analysed		**Uni:** Orphan status, male gender **Multi:** Orphan status	212	logistic regression	400
Kamaya *et al.*[150]‡	Not analysed		**Uni:** male gender, bCD4%<5% **Multi:** male gender, bCD4%<5%	250	logistic regression	400
Jittamala *et al.*[149]‡	Not analysed		**Uni:** male gender, age, adherence **Multi:** none	202	Cox	50
Bienczak *et al.* (this analysis)	C_{12h} > 1.12 mg/L C_{min} > 0.65 mg/L AUC > 28 mg·h/L	C_{12h}, C_{min}, AUC	**Uni:** male gender, age < 8 years, site, WAZ **Multi:** male gender, age < 8 years	118	Cox	100

† target derived based on adult data, ‡ patients treated with nevirapine or efavirenz – presented results relate to efavirenz only, *two efficacy cut-offs used: (A) 400 copies/mL, (B) 50 copies/mL; bCD4% - baseline (pre-ART) CD4 percentage, bVL – baseline (pre-ART) viral load, Cox – Cox proportional hazards regression, IPAM – integrated pharmacokinetic adherence measure, Multi – multivariate analysis, TSSA - tree-structured survival analysis, Uni – univariate analysis, VL – viral load, WAZ – weight-for-age z-score

cannot be excluded and the companion drugs used in the paediatric antiretroviral regimens are different to those used in adults. While the threshold we identified for C12h is not markedly different from 1 mg/mL, our findings do not support dose reduction in children. In our previous analysis we reported that the average exposures across paediatric weight bands dosed according to the current WHO recommendations were above that cut-off.[415] However, the average exposures were significantly affected by *CYP2B6* 516G>T|983T>C genotype and individuals wild type for those polymorphisms are at risk of sub-therapeutic exposures. The results of the current analysis support modifications of the paediatric dosing recommendations based on individual metabolic status.

Among younger children (< 8 years), we found a higher risk of virological non-suppression in boys. Older children (>8 years) has similarly high risk of viral non-suppression in both girls and boys. This phenomenon could arise from differences in treatment adherence by age, as similar effects were observed after adjusting for MEMS adherence. Although the latter is an imperfect measure of adherence, numerous studies have showed that treatment adherence declines with decreasing levels of parental supervision over daily drug intake in older children and adolescents.[417,418] It is less likely that different treatment adherence explains differences between younger boys and girls, in whom caregivers supervise medication intake. Similar effects of male sex were detected in paediatric studies by Janssens[279], Kamaya[150] and Jittamala[149] (Table 5.5).

Adherence measures are cumulative over time since the last visit, whereas PK exposures may be influenced by enhanced pill-taking immediately before clinic visits. In our analysis children who never suppressed had lower average adherence scores and a trend to lower systemic exposures than those who suppressed, a similar trend was identified in the multivariate analysis. In keeping with our findings, Brundage[75] showed that the effect of adherence on the hazard of virological failure was independent of efavirenz exposure.

Children who were treatment-experienced at enrolment were excluded from our main analysis for several reasons. Inclusion criteria required these children to have been on effective antiretroviral treatment for >2 years and have suppressed viral load. It is possible that they therefore had better adherence or were infected with HIV strains free of NNRTI resistance mutations (no pre-ART genotypes were available). They also differed significantly from treatment-naïve children by being older and healthier. Interestingly their PK exposures tended to be higher, supporting a selection effect whereby those with optimal viral suppression are more likely to have higher exposure. All the matched PK/VL samples for this group of children were suppressed and so we could not estimate the subsequent hazard of virological non-suppression.

Our study has several limitations. Most important is the risk of over-fitting the current data when estimating a dichotomised efficacy threshold, with lower external generalisability, which we were unable to test in a validation dataset. The proposed thresholds should also be interpreted in terms of treatment effectiveness in the clinical setting of our study population; their value may be lower in a setting of complete treatment adherence. Adherence in our study was measured only in certain time periods and participants did not use MEMS caps throughout the trial. This intermittent assessment could introduce error into adherence measurement, subsequently affecting the estimated effect of adherence on the risk of non-suppression. Moreover, the wide confidence intervals for efavirenz exposure thresholds predicting a detectable viral load show that larger studies are needed to define thresholds more precisely. We had no viral load data between treatment start and week 36 and therefore could not examine factors affecting time to first suppression, or the impact of PK parameters on VL decline. Furthermore, viral load was measured on average only every 24 weeks so our analysis assumes that no viral rebounds occurred between scheduled measurements.

Despite major concerns, very little CNS toxicity was reported in these predominantly younger children, although this may be more important in adolescents.[419] The relationship between high efavirenz exposures and CNS side effects detected in adults still remains unclear in children.[96–99]

Lastly, antiretroviral therapy consists of a combination of drugs and its efficacy depends on all the components of the tested regimen. Children in CHAPAS-3 were treated with efavirenz and an NRTI backbone consisting of lamivudine combined with either abacavir, stavudine, or zidovudine.[382] Our findings might not be generalisable to different drug combinations, for example, those including more effective companion drugs such as tenofovir, although this is still rarely used in children due to concerns about its impact on growth.

5.6 Conclusions

Efavirenz exposure predicts virological non-suppression, independently of other factors, including adherence, with every two-fold increase in efavirenz concentration reducing the hazard of non-suppression by about 40%. The widely accepted lower therapeutic threshold of 1mg/L for mid-dose concentrations derived in adults is applicable in children, but the cut-offs for trough concentration and $AUC_{0\text{-}24}$ could be lowered to 0.65mg/L and 28mg·h/L, respectively. Our findings should be confirmed in a prospective paediatric trial.

5.7 Acknowledgements

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5.8 Conflicts of Interest and Sources of Funding

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5.9 Appendix to Chapter 5

5.9.1 Supplementary figures

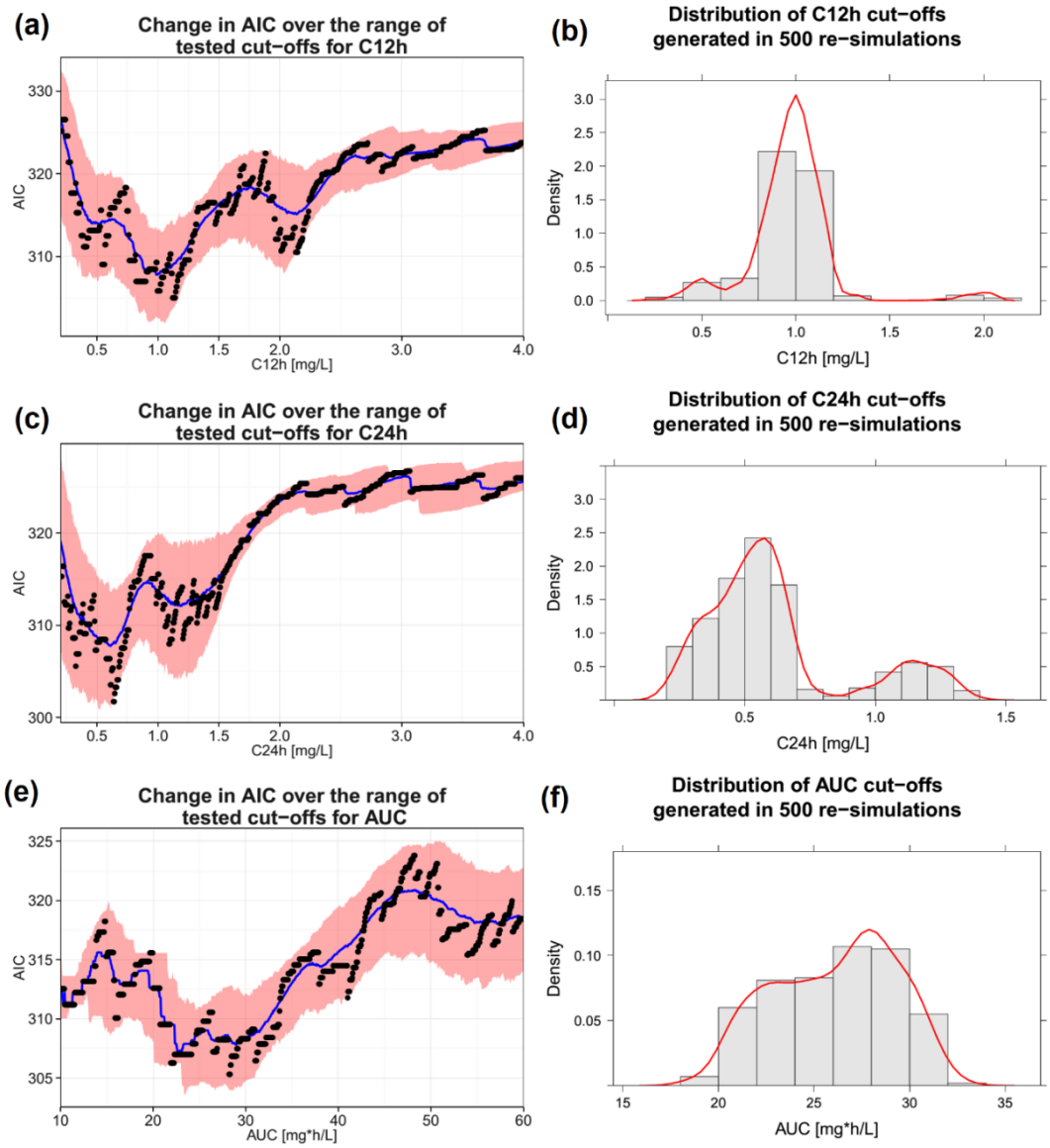

Figure 5.1 Profile likelihoods for virological non-suppression for the range of tested efavirenz exposures and distribution of identified cut-offs

Note: In the left panels the results from the likelihood profiling procedure: the black dots are Akaike Information Criterion (AIC) values for dichotomised cut-offs in tested exposure parameter based on the original data, the blue line and shaded area are the mean and 95% confidence interval of AIC for cut-offs in the tested exposure parameter from 500 re-simulation runs. In the right panels the distribution of the most predictive cut-offs in each of the 500 re-simulation runs. From top to bottom, the results are presented for efavirenz mid-dose concentrations (C12h), trough concentrations (C24h) and AUC – (a) and (b), (c) and (d), (e) and (f), respectively.

CHAPTER 6: THE EFFECT OF DIURNAL VARIATION, CYP2B6 GENOTYPE, AND AGE ON THE PHARMACOKINETICS OF NEVIRAPINE IN AFRICAN CHILDREN.

6.1 Abstract

Objective: We aimed to characterise the effects of *CYP2B6* polymorphisms, diurnal variation, and demographic factors on nevirapine pharmacokinetics in African children.

Methods: Nonlinear mixed-effects modelling conducted in NONMEM 7.3 described nevirapine plasma concentration-time data from 414 children aged 0.3–15 years.

Results: Nevirapine pharmacokinetics was best described using a 1-compartmental disposition model with elimination through a well-stirred liver model accounting for first-pass effect and transit-compartment absorption. Intrinsic clearance was affected by diurnal variation (characterised using a cosine function with peak amplitude 29% at 12 noon) and CYP2B6 metaboliser status (extensive [EM] 516GG|983TT, reference; intermediate [IM] 516GT|983TT or 516GG|983TC, 17% lower; slow [SM] 516TT|983TT or 516GT|983TC, 50% lower; ultra-slow [USM] 516GG|983CC, 68% lower). Age was found to affect pre-hepatic bioavailability: 31.7% lower at birth and increasing exponentially. Median (90% CI) evening C_{min} in the different metaboliser groups were 5.01 (3.01-7.47), 6.55 (3.65-13.32), 11.59 (5.44-22.71), and 12.32 (12.32-27.25) mg/L, respectively. Evening C_{min} were <3mg/L in 43% of EM <6kg and 26% of IM <6kg, while 73% of SM and 88% USM in all weight-bands had evening C_{min} >8 mg/L. C_{min} was not markedly affected by administration time but by unequal splitting of the daily dose.

Conclusions: Diurnal variation does not greatly affect nevirapine exposure. However, when daily doses cannot be split equally, the larger dose should be given in the morning. To achieve homogeneous exposures, nevirapine doses for SM and USM should be reduced by 50%, and children <6kg with EM or IM metabolizer status should receive the same dose as children weighing 6-10 kg.

6.2 Introduction

Nevirapine was the first non-nucleoside reverse transcriptase inhibitor (NNRTI) available in low-income countries in a generic paediatric fixed-dose combination (FDC) tablet. This contributed to substantial cost reductions and improved feasibility of treating HIV-infected children and is still used in resource-limited settings.[33,180,367,371] Nevirapine has several advantageous characteristics: it has fewer drug interactions than protease inhibitors, it does not cause central nervous system (CNS) adverse events when compared to efavirenz, and its bioavailability is not affected by food.[420]

Despite high potency, nevirapine has a low genetic barrier for mutations and suboptimal drug exposures increase risks of developing drug resistance and treatment failure.[341,421] Several studies have reported highly variable nevirapine concentrations, with levels <3 mg/L among children in the lower paediatric weight-bands when dosed according to WHO guidelines increasing their risk of virological failure.[33,37,77,305,367,368,372] Nevirapine concentrations >8mg/L on the other hand were associated with an increased risk of treatment discontinuation due to adverse events among adults.[341] However, paediatric studies quantifying nevirapine pharmacokinetic variability due to different sources and suggesting optimisation of current dosing remain limited.[278,305,374]

Nevirapine has complex metabolism mediated mainly by *CYP3A4* and *CYP2B6* coded enzymes.[301] Single nucleotide polymorphisms (SNPs) present in *CYP2B6* - 516G>T and 983T>C were identified as the main source of nevirapine variability in adults,[72,73,198] as for efavirenz.[64,180,198] The prevalence of 516G>T loss of function (LOF) polymorphisms differs between populations and is particularly high in black Africans, whereas 983T>C variants are not observed among Caucasians.[64,180,198] In our previous investigation of efavirenz pharmacokinetics (PK) in African children, we showed that extensive metabolisers (*CYP2B6* 516GG|983TT genotype), are at higher risk of developing sub-therapeutic efavirenz concentrations.[415] A similar investigation of differences in nevirapine exposures between various metaboliser groups when dosed by weight-band according to current WHO guidelines has not yet been conducted in children. *CYP2B6* expression may be further modified by polymorphisms in genes coding nuclear receptors CAR (NR1|3) and PXR (NR1|2),[78,81] although this has not been proved for nevirapine.[422]

The effect of the *CYP3A4* pathway on nevirapine PK is less studied. Although not confirmed for nevirapine, systemic exposures of *CYP3A* substrates have been shown to be altered by SNPs rs35599367 (*CYP3A4**22)[85,86] and rs776746 (*CYP3A5**1).[87,88] Additionally, *CYP3A* activity exhibits diurnal variation with clearance rates increased during the day and reduced at night.[89,90] Differences between morning and evening nevirapine trough concentrations (C_{min}) have been previously reported [91] and may relate to diurnal variation in *CYP3A*-mediated effects on PK.

The aim of this analysis was to: (i) model the steady-state population PK of nevirapine in the largest cohort of African children studied so far, (ii) quantify demographic and genotypic effects on nevirapine disposition, (iii) characterise the effect of diurnal variation on nevirapine exposures under various dosing scenarios, and (iv) propose optimal dosing strategies for this population.

6.3 Methods

In this analysis, sparsely sampled data from the CHAPAS-3 trial (Children with HIV in Africa - Pharmacokinetics and Adherence of Simple Antiretroviral Regimens)[382] was enriched with intensive data from an earlier PK sub-study[33] (part of CHAPAS-1).[107] Both studies were conducted in African children from Uganda and Zambia, as briefly described below.

6.3.1 CHAPAS-1

The trial evaluated dosing of, and adherence to, new paediatric FDC tablets: Triomune Baby (nevirapine 50mg, stavudine 6mg, lamivudine 30mg) and Junior (nevirapine 100mg, stavudine 12mg, lamivudine 60mg) dosed twice-daily according to WHO 2006 guidelines in children <14 years.[56] When the daily dose could not be split equally, the larger dose was given at night.

Children in the PK sub-study were sampled on one occasion at least 4 weeks after starting treatment. Samples were taken immediately before the observed morning dose and 1, 2, 4, 6, 8 and 12h subsequently. The time of the preceding evening dose was assumed to be 12h before the morning dose. Samples were stored and assayed using ultra high-performance liquid chromatography with UV detection at the Department of Pharmacy of the Radboud University Medical Centre, Nijmegen, Netherlands. The method was linear over the range of 0.1-10 mg/L. The average intra-assay and inter-assay coefficients of variation (CV) and relative error (RE) were 2.9%, 2.4%, and 97%, respectively.[423]

6.3.2 CHAPAS-3

PK, toxicity, acceptability, adherence, and virological efficacy were compared between three first-line antiretroviral regimens in children 13 years or younger. [382] Depending on treatment allocation, patients received: Triomune Baby, Triomune Junior, Duovir-N Baby (nevirapine 50mg, zidovudine 60mg, lamivudine 30mg) or nevirapine (100mg) – all paediatric formulations; or Duovir-N (nevirapine 200mg, zidovudine 300mg, lamivudine 200mg) or Triomune30 (nevirapine 200mg, stavudine 30mg, lamivudine 150mg), formulated for adults. Nevirapine-based regimens were dosed twice daily according to WHO 2010 guidelines.[11] When the daily dose could not be split equally, the larger dose was given in the morning.

Children on nevirapine were sampled during clinic visits at weeks 6, 36, and every 24 weeks thereafter until the end of the study; at each visit 2 samples were taken at least 2h apart. The self-reported time of the morning and penultimate doses were recorded. Samples were stored and analysed by liquid chromatography-tandem mass spectrometry at the Division of Clinical Pharmacology, University of Cape Town, South Africa. The method was linear over the range of 0.0195-20 mg/L. The average intra-assay and inter-assay CV and RE were 2.9%, 2.4% and 97%, respectively.

6.3.3 Genotyping

Genotyping was performed on patients from CHAPAS- 3 only, by allelic discrimination real-time PCR assay on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The PCR protocol involved an initial denaturation step at 95°C for 15 min, followed by 50 cycles of amplification at 95°C for 15 s and final annealing at 60°C for 1 min. TaqMan® Genotyping Master Mix and assays for CYP2B6 516G>T (rs3745274; ID: C_7817765_60), CYP2B6 983T>C (rs28399499; ID: C_60732328_20), CYP2B6 15582C>T (rs4803419; ID: C_7817764_10), CYP3A4*22 (rs35599367, C__59013445_10), CYP3A5 6986G>A (rs776746, C__59013445_10), NR1I3 (rs3003596, C__16194070_10 and rs2307424, C__25746794_20), NR1I2 63396C>T (rs2472677, C__26079845_10), ABCC10 (rs2125739, C__16173668_10) were obtained from Life Technologies Ltd (Paisley, UK). Opticon Monitor® version 3.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to obtain allelic discrimination plots and make allele calls.

The distribution of the genotypes was tested for Hardy-Weinberg equilibrium using the exact test in the R 'genetics' package.

6.3.4 Population pharmacokinetic analysis

6.3.4.1 Model building

The steady-state PK of nevirapine was analysed using nonlinear mixed-effects modelling with NONMEM 7.3[408] and the first-order conditional estimation method with interaction. PsN 4.4.0, Pirana, and Xpose were used to facilitate modelling and for model diagnostics.[379] Model building was conducted starting with intensive PK data from CHAPAS-1 followed by sparse from CHAPAS-3.[294] The stepwise process was guided by differences in NONMEM objective function value (OFV; proportional to -2 log-likelihood), inspection of goodness-of-fit (GOF) plots and visual predictive checks (VPCs), biological plausibility, and clinical relevance. OFV drops >3.84 between two hierarchical models after adding one parameter were considered a significant improvement ($P \leq 0.05$, χ^2-distribution, df=1). Stability and robustness of the final model, together with precision of parameter estimates, was evaluated using nonparametric bootstrap (n=50, due to long model run times).

The model-derived Empirical Bayesian Estimates for the individual parameters were used to predict morning (AM) and evening (PM) C_{min} and AUC_{0-12} (area under the concentration-time between dosing events) at steady-state for each sampling occasion and patient.

6.3.4.2 Structural model

1- , 2-, and 3-compartment disposition models with first-order absorption and elimination were tested, as well as delayed and transit comparment[410] absorption. A semi-mechanistic well-stirred hepatic extraction model was tested for elimination, as in Gordi *et.al*[411]. The hepatic model assumed nevirapine fraction unbound in plasma (f_u) 40%,[292] hepatic plasma flow (Q_H) 50 L/h,[424] and liver volume (V_H) 1L[411] for a typical 70 kg individual (allometrically scaled).

Between-subject variability (BSV) and between-occasion variability (BOV) were tested on all PK parameters assuming lognormal distribution. Residual unexplained variability (RUV) was described using a combined proportional and additive structure. We excluded from the analysis data with uncertain dosage history and nevirapine concentrations below-limit-of-quantification (BLQ), presumed to be due to non-compliance[294] (confirmed by undetectable concentrations of the companion antiretroviral drugs). Further implausible outliers were identified using visual checks and excluded based on conditional weighted residuals (|CWRESI|>3).

6.3.4.3 Covariate effects

Allometric scaling was added to the model at an early stage (before covariate testing), as suggested by Holford *et al.*[225] and applied to all clearance and volume parameters. For intrinsic clearance (CL_{int}) and pre-hepatic bioavailability (F_{preH}) we tested the effect of age using a power- , hockey-stick- , exponential- or sigmoidal function with/without Hill coefficient-models[225]. The effect of diurnal variations was investigated using step or cosine functions.[90] Besides weight and age, the other covariates tested were: study site, nucleoside reverse transcriptase inhibitor (NRTI) treatment backbone, sex, weight-for-age Z-score (WAZ), height-for-age Z-score (HAZ), and formulation. Pharmacogenetic effects were tested as individual SNPs (rs3745274, rs28399499, rs4803419, rs35599367, rs776746, rs3003596, rs2307424, rs2472677, rs2125739) and as metaboliser status determined by SNPs 516G>T and 983T>C (extensive [EM], genotype 516GG|983TT; intermediate [IM], single variant allele [516GT|983TT or 516GG|983TC]; slow [SM], 2 variant alleles [516TT|983CC or 516GT|983TC]; ultra-slow [USM], 983CC irrespective of 516G>T genotype).

Mixture modelling with frequencies fixed to those observed in study population was used to impute missing genotypes (predominantly in CHAPAS-1).[413] Proportionality and correction factors were applied on RUV to test for differences between the assays and laboratories used.

6.3.4.4 Simulations

For simulation (conducted with NONMEM 7.3) demographics from the 414 patients (weight 3.5 - 29.6 kg) from the original analysis were used and enriched with 116 records of individuals weighing 20-35 kg from CDC Growth Charts (age and corresponding median weight used).[425] The final model was used to simulate exposures after nevirapine administration under various dosing scenarios and assuming 3–8 mg/L as therapeutic range for nevirapine.[95] Each *in silico* patient was re-simulated 100 times, changing their metaboliser status according to the proportions in the study population, which ensured the same distribution in each weight-band. The effect of drug intake time (6:00, 7:00, 8:00, 9:00 AM/PM) and dose-splitting strategies (AM/PM D1:100/50 mg, D2:75/75 mg, D3:50/100 mg) was studied in a single patient (0.44 years, 7.2 kg, IM) simulated 1000 times. To avoid generating implausibly extreme values, the maximum variability for each random effect was limited to 3 standard deviations. Data analysis and plots generation was performed using R[380].

Table 6.1 Demographic characteristics of children in CHAPAS-1 and CHAPAS-3 treated with nevirapine

Characteristics		CHAPAS-1	CHAPAS-3	Combined
No. of children		84	336	414
No. of samples included		539	2766	3305
No. of samples excluded (BLQ)		8 (0)	238 (48)	246*
No. of sampling occasions		1	3 (1-7)	3 (1-8)
Age [years]**		6.2 (0.4-15.0)	2.6 (0.3-12.2)	2.92 (0.3-15.0)
Weight [kg] **		15.75 (3.5-29.0)	11.5 (4.9-29.6)	12.2 (3.5-29.6)
WAZ		-1.1 (-4.2 – 2.0)	-1.7 (-7.2 – 1.2)	-1.5 (-7.2 – 2.0)
Sex [M/F]		52/32	177/159	80/89
NRTI	**ABC**	0	115	115
	d4T	84	107	191
	ZDV	0	114	114

Note: Data are number of subjects or median (range); *Samples excluded from the analysis: unclear dosage history – 111, implausible (visual check confirmed by |CWRES|>3) – 87, BLQ confirmed by undetectable levels of the companion drugs – 48. **Baseline values. BLQ – below limit of quantification; WAZ - weight-for-age Z-score, M – male; F – female; ABC – abacavir; d4T – stavudine; ZDV – zidovudine.

Among presented patients 6 rolled over from CHAPAS-1 to CHAPAS-3. All patients were black Africans.

6.4 Results

6.4.1 Demographic characteristics and samples

This analysis included 3305 samples (539 in intensive and 2766 in sparse PK profiles) from 414 African children (78 CHAPAS-1, 330 CHAPAS-3, 6 in both). Baseline demographic characteristics are presented in Table 6.1. Genotypes were available for 324 children (Table 6.4 in Appendix to Chapter 6); *CYP2B6* metaboliser groups were 33.1% EM, 44.6% IM, 21.7% SM, 0.6% USM (Table 6.2); the mixture-model allocation for remaining 96 individuals was: 41.7% EM, 49.0% IM and 9.4% SM. All tested genotypes were in Hardy-Weinberg equilibrium (Table 6.4 in Appendix to Chapter 6). 246 samples were excluded from the analysis (111 due to unclear dosage history, 87 outliers, and 48 BLQ).

6.4.2 Population pharmacokinetics

Nevirapine pharmacokinetics was best described using 1-compartment disposition, absorption through transit compartments, and elimination using the semi-physiological model with 1st-pass hepatic extraction (Figure 6.1 and Chapter 6.9.1 in Appendix to Chapter 6). The final model parameters were estimated relative to pre-hepatic bioavailability (F_{preH}, with typical value fixed to 1) and are presented in Table 6.3. All parameter estimates were found to be reasonably robust and adequate model fit was confirmed through VPC and GOF plots, which showed adequate fit of our model to the analysed data (Figure 6.2 and Figure 6.6 [in Appendix to Chapter 6]).

Implementing the well-stirred liver model decreased OFV by 42, without adding extra parameters. The model was parameterised with CL_{int} following a circadian rhythm expressed through oscillations of the cosine function with zenith around 12 noon and amplitude of approximately 29% (ΔOFV=-91, df=2, P<0.001) (Figure 6.3). The model identified distinct pre-hepatic (F_{preH}) and hepatic components (F_H) of bioavailability, since changes in liver activity mechanistically affected also F_H. The reference value of F_{preH} was fixed to 1, and BSV and BOV were estimated. Including the diurnal effect reduced BSV in CL_{in} by 34% and BOV in F_{preH} by 41%. More details on the model implementation, including formulae explaining relationship between model parameters, are presented in the Chapter 6.9.1 in Appendix to Chapter 6

Table 6.2 Nevirapine exposures of different metabolic subgroups determined by 516GT|983TC SNP vector

Metaboliser Status	Pts	C_{minAM} [mg/L]	C_{minPM} [mg/L]	$C_{minPM} < 3$ [mg/L]†	$3 < C_{minPM} < 8$ [mg/L]†	$C_{minPM} > 8$ [mg/L]†	AUC_{AM} [mg·h/L]	AUC_{PM} [mg·h/L]
EM	106 (33.3%)	5.01 (3.01-7.47)	4.58 (2.53-7.03)	77 (16.6%)	361 (77.6%)	27 (5.8%)	68.51 (39.42-104.16)	69.34 (38.65-104.42)
IM	141 (44.2%)	6.55 (3.65-13.32)	6.08 (3.25-12.93)	33 (5.8%)	378 (66.8%)	155 (27.4%)	88.93 (50.06-173.72)	88.60 (50.06-173.72)
SM	70 (21.9%)	11.59 (5.44-22.71)	10.91 (5.06-22.44)	4 (1.3%)	78 (25.7%)	222 (73.0%)	152.07 (72.42-270.46)	151.27 (71.54-287.46)
USM	2 (0.6%)	12.32 (12.32-27.25)	11.71 (11.71-26.43)	0 (0%)	1 (12.5%)	7 (87.5%)	170.81 (170.81-362.97)	152.12 (152.12-337.26)

Note: Data are median (5^{th}-95^{th} percentile) or number (percentage). †Number of C_{min} below/within/above the therapeutic range of 3 – 8 mg/L.[45] EM (extensive metabolisers) - 516GG|983TT; IM (intermediate metabolisers) - 516GG|983TC or 516GT|983TT, SM (slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (ultra-slow metabolisers) - 516GG|983CC.

Data for 319 individuals from CHAPAS-3 trial with available genotype dosed according to WHO 2010 guidelines[11] corresponding to 1343 PK visits. When multiple PK visits were available, measurements were used to calculate the geometric mean for every patient, which were then used to calculate median and percentiles in each subgroup.

After applying allometric scaling to account for the effect of body size, and including diurnal effects and 1st-pass metabolism, the most significant covariate was the metaboliser status on CL_{int} determined by *CYP2B6* 516G>T|983T>C genotype (ΔOFV=-217, df=3, P<0.001), explaining 85% of remaining BSV in CL_{int}. Using six rather than four 516G>T|983T>C SNP-vector metaboliser groups[415] reduced OFV by only 5 points (df=2, P=0.08) and was therefore not used.

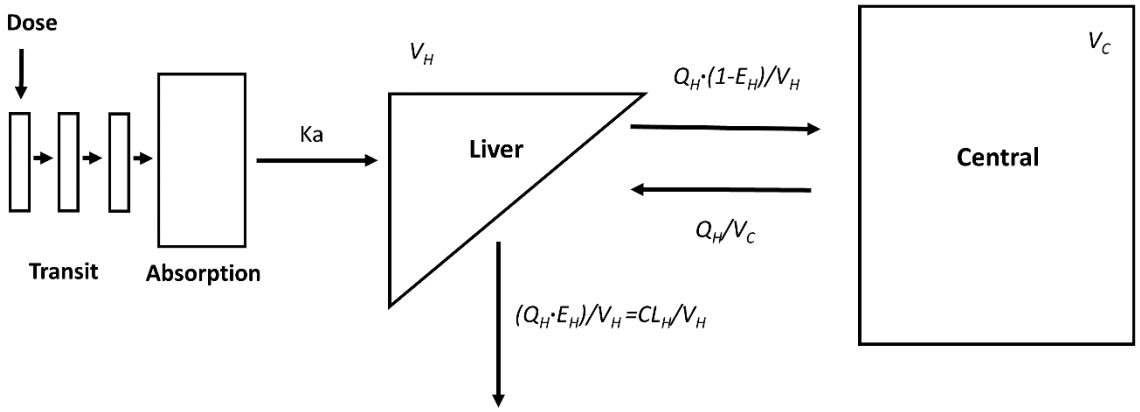

Figure 6.1 Compartmental structure of the nevirapine pharmacokinetic model.

Note: CL_H – hepatic clearance; E_H – hepatic extraction; Ka – absorption rate constant; Q_H – hepatic plasma flow; V_H – volume of the liver; V_C – volume of the central compartment. The model parameters and presented relations are explained in detail in the Appendix to Chapter 6..

Our data did not support a maturation effect on CL_{int}, but we identified age-driven differences in F_{preH}, which were described using an exponential model (equation 7 in the Appendix in the Supplement). F_{preH} at birth was estimated as 58.3% of the value in older children (reference fixed to 100%), 90% of F_{preH} was reached by age of approximately 3.3 years and the half-life of the process was 1.55 years (Figure 6.4).

The model estimated that an average child weighing 14.5 kg and aged 4.1 years would have F_{preH}=93% and their values of oral clearance (CL_{oral}, see Chapter 6.9.1 and Figure 6.5 in Appendix to Chapter 6) were 1.31 L/h EM (reference), 1.09 L/h IM (17% lower), 0.66 L/h SM (50% lower) and 0.42 L/h USM (68% lower). A summary of the individual exposures in children from CHAPAS-3 trial dosed according to WHO 2010 guidelines[11] is presented in Table 6.2, split by metaboliser genotype.

*Table 6.3 Final nevirapine population parameter estimates (5th and 95th percentile)**

Parameter		Typical values	Variability
CL_{int}	EM [L/h]	3.27 (3.00 – 3.69)	BSV: 21.40 (20.08 – 32.46)
	IM [L/h]	2.72 (2.27 – 2.94)	
	SM [L/h]	1.65 (1.47 – 1.89)	
	USM [L/h]	1.04 (0.87 – 1.38)	
	AMP [%]	29.2 (27.7 – 45.2)	
	SHIFT [h]	-12.30 (-13.32 – -10.38)	
V_c [L]		21.92 (20.24 – 26.23)	
F_{preH}	Older Children[ϯ]	1 (FIXED)	BSV: 18.72 (6.59 – 20.66) BOV: 17.02 (16.12 – 20.87)
	At Birth [%]	58.30 (50.48 – 68.24)	
	$t_{1/2}$ [years]	1.54 (1.47 – 2.58)	
MTT [h]		0.56 (0.49 – 0.70)	BOV: 199.73 (177.23 – 217.70)
KA [1/h]		0.84 (0.67 – 1.12)	BOV: 44.91 (31.32 – 50.46
N_{TRANS} [number]		3 (FIXED)	
Increased BOV F_{preH} for unobserved intake		1.54 (1.20 – 1.65)	
Additive error [mg/L]		0.32 (0.21 – 0.38)	
Proportional error [%]		5.26 (4.26 – 6.18)	
Increased error for sparse data		1.56 (1.49 – 1.81)	

Note: Final parameter estimates are typical population values estimated by the model. All clearance and volume parameters scaled allometrically to the median weight of 14.5 kg. *Estimated from nonparametric bootstrap (n=50) of the final model. ϯOlder children refers to individuals where no further age-driven increase in bioavailability can be observed (Figure 6.4). CL_{int} – intrinsic clearance; EM – extensive metaboliser; IM - intermediate metaboliser; SM – slow metaboliser; USM – ultra-slow metaboliser; AMP – amplitude of cosine function; SHIFT – shift in the zenith of cosine function from midnight; Vc –volume of central compartment; FpreH – pre-hepatic bioavailability; NTRANS – number of transit compartments (in the implementation as Savic et al.39 this would be NN=2) MTT – absorption mean transit time; Ka – absorption rate constant; BSVCL - between subject variability in clearance intrinsic; BSV – between-subject variability; BOV – between-occasion variability

The number of transit compartments was first estimated and then fixed during the covariate analysis in order to improve model stability. The number was then re-estimated in the final model and proved not to be different from previously fixed.

Higher uncertainty related to unobserved intake time (for all sparse data and pre-dose samples in intensive data) was accounted for by scaling factors (proportional model) on RUV and BOV F_{preH}, which were found to be respectively 1.56 and 1.54 times larger than in PK samples after observed intake.

No other covariates were identified as significant. The remaining stochastic variability in clearance and bioavailability was low (BSV CL_{int} 21.4%, BSV F_{preH} 18.7% and BOV F_{preH} 17%), but absorption parameters (where no covariates improved model fit) remained highly-variable (BOV absorption rate constant [KA] 44.9%, BOV absorption mean transit time [MTT] 199.7%).

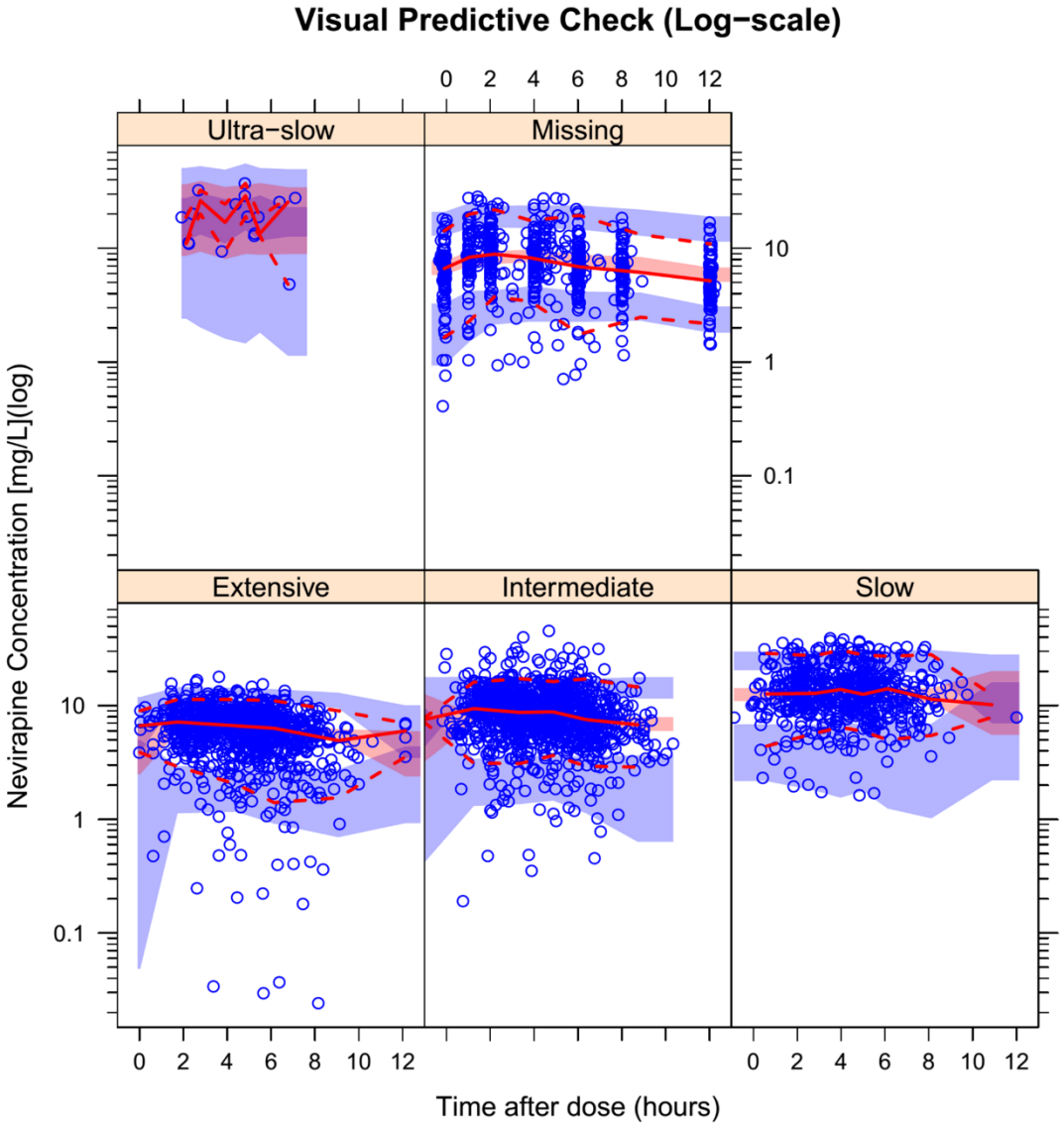

Figure 6.2 Visual Predictive Check of the final nevirapine population pharmacokinetic model by metabolic status (determined by combined effect of SNPs 516G>T and 983T>C) in semi-log scale.

Note: Hollow dots – observed concentrations, red solid line – median of observed data, red dashed line– 5th and 95th percentile of observed data, pink shaded area – 95% confidence interval of simulated median, blue shaded area - 95% confidence interval of simulated 5th and 95th percentile. For explanation of metabolic subgroups see Methods (Chapter 6.3.4.3).

6.4.3 Simulations

Simulations were conducted to compare average C_{minAM} and C_{minPM} in weight-bands of African children divided into metaboliser groups and dosed following WHO 2010 recommendations.[11] Average C_{minAM} and C_{minPM} in weight-bands >6kg were >3 mg/L for most simulated individuals regardless of metaboliser status (Figure 6.5a). In contrast, >25% of children in the lowest weight-band (4-6kg) had C_{minPM} below the efficacy threshold (Figure 6.5b); this effect was driven mostly by EM and IM (43% and 26% <3mg/L, respectively).

Given the detected diurnal variation in nevirapine CL_{int} we evaluated the effect of administration time (see Methods) on average morning and evening exposures. The change in median concentration depending on administration time and differences in systemic drug exposures are presented in Figure 6.7 in Appendix to Chapter 6. Depending on administration time, the ratios of AM/PM exposures varied between 1.09–1.15 for C_{min} and 1.03–1.07 for $AUC_{0\text{-}12}$, differences of unlikely clinical significance.

Use of some nevirapine FDCs can lead to unequal splitting of the advised daily dose between morning and evening intakes. Simulation results showed that ratios between simulated median C_{min} AM/PM for tested dose-splitting strategies (see Methods) were: D1 (larger morning) 0.93, D2 (equal) 1.13, D3 (larger evening) 1.41; and $AUC_{0\text{-}12}$ 0.90, 1.04, and 1.22, respectively (Figure 6.8 in Appendix to Chapter 6).

6.5 Discussion

We present the largest investigation to date of nevirapine pharmacogenetics, the first report of the effect of 983CC homozygosity on nevirapine PK and the first study in children to quantify the combined effect of *CYP2B6* 516G>T|983T>C. Our analysis is also the first to date to characterise the diurnal variation in nevirapine clearance through population pharmacokinetic modelling and to evaluate the effect of this phenomenon on systemic drug exposures through simulations.

The main predictor of nevirapine clearance in our cohort of African children was the combined effect of *CYP2B6* 516G>T|983T>C genotype. Oral clearance estimated by our model before adjusting for the *CYP2B6*-SNPs was 3.8 L/h, which was comparable with 3.93 L/h reported previously in children[374] (both scaled up to 70kg) and 2.82–3.97 L/h in adults.[50,66,72,73,294,295,297,298,378] Comparing the *CYP2B6* 516G>T|983T>C effect with other reports is problematic, since our study is the first to use this categorisation with four metaboliser subgroups for nevirapine, although it has been extensively

applied to efavirenz.[71,415] The 50% lower nevirapine clearance we detected for SM is greater than the 30-37% drop previously reported for 516TT versus 516GG.[72,73,105,278,300,305] Similar to efavirenz,[198,415] the effect of *CYP2B6* 983CC (recessive homozygosity) on nevirapine PK has greater magnitude than 516TT (68% versus 50% drop). Unsurprisingly, nevirapine clearance is affected to lesser degree by *CYP2B6*-polymorphisms than efavirenz in the same population.[415] This can be explained by a different contribution of the CYP3A4 pathway to the metabolism of these drugs.[177]

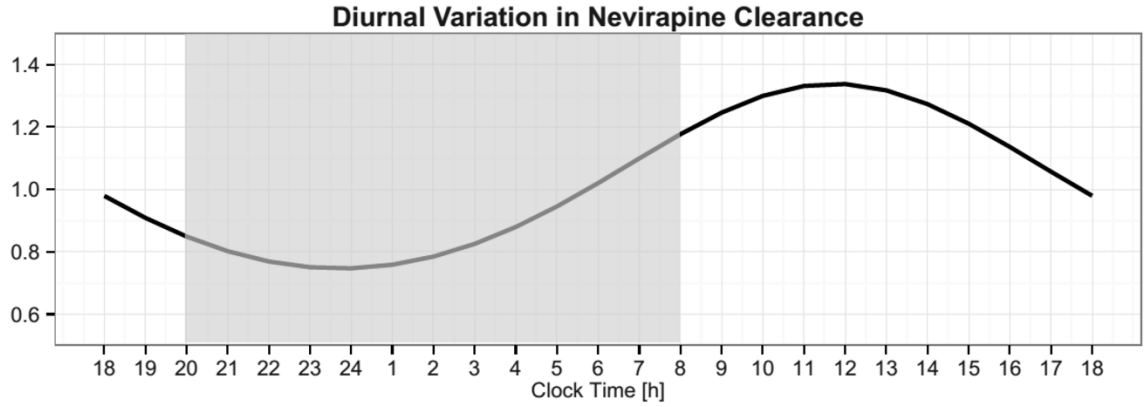

Figure 6.3 Diurnal variation in nevirapine intrinsic clearance detected by the model, presented over 24 h.

Note: Shaded area refers to night (20h00 – 08h00)

Diurnal variation has been previously documented for several CYP3A4 substrates,[426,427] consistently revealing increased clearance rates during the day as compared to during the night.[89,322,428] Our study replicated those findings and detected significantly higher nevirapine clearance during the day with maximum at midday. The estimated amplitude of the diurnal variation is somewhat larger than previous reports in CYP3A4 probes.[89,428] This could be due to the considerable contribution of CYP2B6 enzymes to nevirapine clearance. Although little is known about chrono-pharmacokinetics of this pathway, our hypothesis is supported by the fact that CAR, which regulates expression of *CYP2B6*,[78] exhibits a circadian rhythm linked to a 1.7-fold magnitude induction of *CYP2B* mRNA.[429]

Despite the 29% amplitude of diurnal variation in nevirapine clearance, the simulated difference between morning and evening trough exposures was less than 15%. This lack of effect is due to nevirapine's relatively long half-life (25-30h at steady-state)[292] in comparison to, for example, protease inhibitors, where the reported median difference in troughs are almost 60%.[322] Simulations revealed only a marginal effect of intake time on exposures, but showed that the diurnal variation should be considered when the daily dose of nevirapine cannot be split equally, since 50% difference in the ratio of median C_{min} AM/PM was found depending on whether the larger dose is given in the morning or

evening. To minimise this effect and the risk of suboptimal exposures, uneven splitting should be implemented with the larger dose given in the morning, which is currently not specified in the WHO guidelines.[11]

Further novelty of our study was the use of a semi-physiological well-stirred liver model allowing the effect of hepatic clearance (expressed as clearance intrinsic) on both systemic clearance and 1st-pass hepatic extraction to be captured, so that clearance and its covariates affect bioavailability. This model allowed us to separate the pre-hepatic and hepatic components of bioavailability.

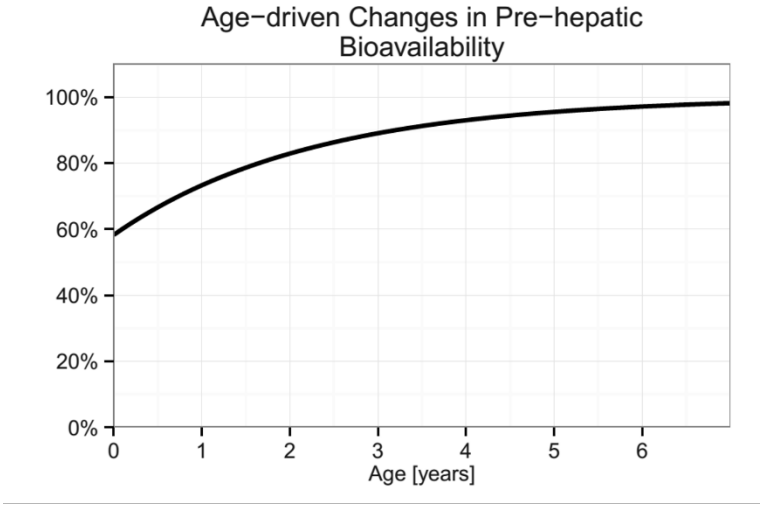

Figure 6.4 Change in nevirapine pre-hepatic bioavailability with age.

A significant degree of variability in nevirapine pharmacokinetics was explained in our model by age-driven differences in pre-hepatic bioavailability, which possibly overshadowed the expected effect of maturation of the metabolic pathways. A similar effect was found for nevirapine by Foissac *et al.*[374] and reported for other antiretroviral drugs[13] and could hypothetically be caused by reduced drug absorption in neonates and younger children. This may be due to more rapid gastric emptying, smaller gastric volume, higher gastric pH, smaller gastro-intestinal absorption area, as well as adherence and palatability issues.[430] This phenomenon could explain sub-therapeutic concentrations seen in the youngest age groups in other paediatric studies.[33,37,77,305,367,368,372] Our simulations show in particular that individuals in the <6 kg weight-band who are EM and IM are at risk of suboptimal exposures (observed PM C_{min} <3 mg/L in 43% and 26% of individuals, respectively).

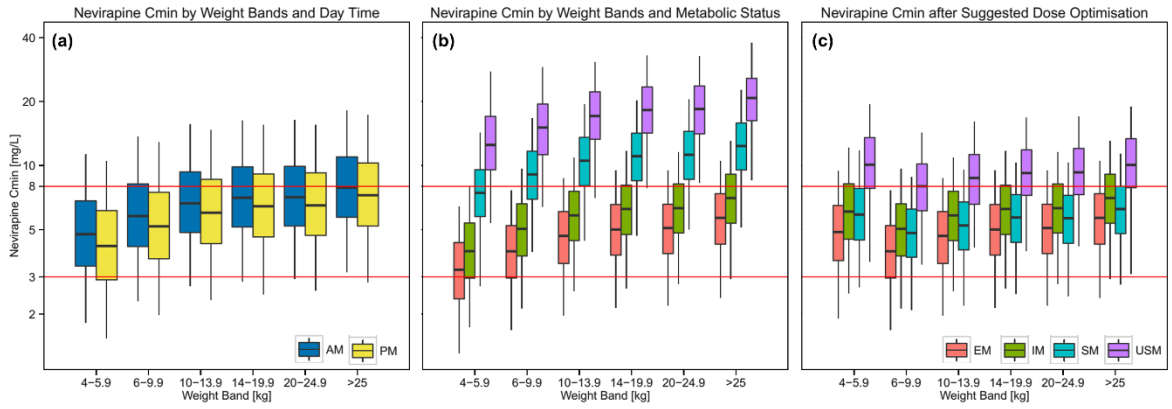

Figure 6.5 Model-simulated nevirapine exposures shown by dosing weight bands and metabolic subgroups

Note: Presented plots show: (a) difference between morning and evening nevirapine C_{min} when dosed according to current WHO recommendations;[11] (b) difference in nevirapine C_{min} between metabolic groups when dosed according to current WHO recommendations (evening C_{min} is shown); (c) difference in nevirapine C_{min} between metabolic groups when dosed according to proposed dose optimisation strategy (evening C_{min} is shown). Red horizontal lines correspond to nevirapine therapeutic range, form 3 mg/L to 8 mg/L.[95] The box in the percentile plots shows the 25th, median, and 75th percentile, while whiskers correspond to 5th and 95th percentile of the simulated data. EM (extensive metabolisers) - 516GG|983TT; IM (intermediate metabolisers) - 516GG|983TC or 516GT|983TT, SM (slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (ultra-slow metabolisers) - 516GG|983CC.

Despite significant differences in nevirapine PK determined by *CYP2B6* genotype, a genotype-driven dose optimisation strategy has not been previously suggested. This could be due to the fact that, unlike efavirenz, the relationship between high exposures and toxicity is not strongly apparent.[72,234,341] Nonetheless, suboptimal concentrations are of concern, as they could lead to virological failure.[37,305,341,374] To prevent suboptimal exposures we suggest the dose for EM and IM in the lowest weight-band be increased from 100mg to 150 mg. Further harmonisation of exposures across metaboliser groups could be achieved by 50% reduction of nevirapine dose for SM and USM in all other weight-bands, as >75% of those children had PM C_{min} above the 8 mg/L therapeutic upper-limit, although this might be of limited clinical relevance. When daily dose cannot be split equally, larger doses should be given AM. The simulated C_{min} based on this dose optimisation approach are presented on Figure 6.5c. We acknowledge however that practical implementation of such strategy in resource-limited settings would be hindered by restricted access to genotyping and current use of FDCs.

Our study has several limitations. The therapeutic range for nevirapine used in our analysis has not been previously evaluated in children or African populations. The intake time for sparse PK data was self-reported and might be inaccurate, given the large variability in absorption parameters, and could

inflate the magnitude of the detected diurnal variation. We tried to minimise this effect by excluding samples with uncertain dosage information and BLQ. The detected diurnal effect could hypothetically be further affected by food intake, which was not recorded in our study. However, food has been previously reported not to modify nevirapine bioavailability or clearance.[420] Additionally, the analysed trials differed in the AM/PM dose splitting strategy (see Methods), but the model-based approach we employed accounts for this difference.

6.6 Conclusions

This is the first study quantifying the combined effect of *CYP2B6* 516GT|983TC on nevirapine clearance in children and classifying metabolisers into four metabolic groups (extensive [EM], intermediate [IM], slow [SM], ultra-slow [USM]). To prevent sub-therapeutic exposures EM and IM children <6kg should receive same the dose as those in the 6-10 kg weight-band. Further homogenisation of exposures can be achieved reducing the current recommended dose for SM and USM by 50% in other weight-bands. Additionally, we characterised the effect of diurnal variation on nevirapine pharmacokinetics, and found that it is of limited clinical relevance, possibly due to nevirapine long half-life. However, this phenomenon should be taken into consideration when daily doses cannot be split equally and larger doses should be given in the morning.

6.6 Acknowledgements

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6.7 Funding statement

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6.8 Transparency declarations

All authors have completed the Unified Competing Interest form and declare: AB, AC, VM, CK, AK, ASW, DMG, HM and DB received support through grants from European Developing Countries Clinical Trials Partnership (EDCTP); AC, AK, ASW and DMG additionally received grants from Medical Research Council (MRC) UK; HM additionally declares support in part by the National Research Foundation of South Africa, grant 90729; AO received support in form of grants from Janssen, ViiV and Tandem Nano, as well as personal fees from Merck was issued a patent “Compositions of efavirenz”. All other authors: none to declare. No other support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work are to be declared for any of the authors.

6.9 APPENDIX TO CHAPTER 6

6.9.1 Description of the nevirapine semi-mechanistic model with 1st pass metabolism

The nevirapine model presented in Figure 6.1 is explained in detail below.

The delay between oral administration and absorption is modelled through 2 transit compartments. After entering the absorption compartment, nevirapine is transferred to the liver, where it undergoes 1st-pass hepatic extraction (E_H). The fraction of the drug not eliminated by 1st-pass (1-E_H) is then transported via hepatic plasma flow (Q_H) to the central compartment and the systemic circulation. It then recirculates back to the liver, which is the site of drug clearance. In this well-stirred model, hepatic clearance (CL_H) is determined by Q_H and E_H as follows:

(1) $$CL_H = Q_H \cdot E_H$$

E_H depends on the unbound fraction of the drug (f_u), liver activity (CL_{int}), and Q_H and is defined as:

(2) $$E_H = \frac{CL_{int} \cdot f_u}{CL_{int} \cdot f_u + Q_H}$$

E_H also determines the hepatic bioavailability F_H

(3) $$F_H = 1 - E_H$$

The total oral bioavailability (F) is determined by both the pre-hepatic (F_{preH}) and hepatic (F_H) components, as follows:

(4) $$F = F_{preH} \cdot F_H$$

After a number of transformations, oral clearance can be simplified as follows:

(5) $$CL_{oral} = \frac{CL}{F} = \frac{CL_{int} \cdot f_u}{F_{preH}}$$

Due to circadian rhythm variations, CL_{int} changes with time, thus affecting both CL_H and F_H, and its value at time (t) is defined as follows:

(6) $$CL_{int}(t) = CL_{int} \cdot e^{AMP \cdot cos\left(\frac{2\pi}{24} \cdot (t - SHIFT)\right)}$$

where AMP is the amplitude of the cosine oscillation and SHIFT is the phase shift of the cosine function relative to 00:00. In order to prevent negative values of CL_{int} the effect of the circadian rhythm was modelled as exponential and can be interpreted approximately as a relative change.

Furthermore, F_{preH} changes with age, as expressed by following equation:

(7) $$F_{preH} = 1 - \left(1 - F_{preH\_BIRTH}\right) \cdot e^{-K_{FpreH} \cdot AGE}$$

where F_{preH_BIRTH} is the F_{preH} at birth, K_{FpreH} is the rate constant for age-driven change in F_{preH} and AGE refers to age.

6.9.2 Supplementary tables

Table 6.4 Observed frequencies of tested single nucleotide polymorphisms with porresponding Hardy-Weinberg P-values in children from CHAPAS-3 treated with nevirapine

Gene	SNP	Hom-Ref†	Het-LOF†	Hom-LOF†	MAF	HWE P-value
CYP2B6	**rs3745274 (516G>T)**	GG	GT	TT	0.36	0.18
		136 (0.43)	136 (0.43)	47 (0.15)		
	rs28399499 (983T>C)	TT	TC	CC	0.09	1
		226 (0.83)	51 (0.16)	2 (0.01)		
	rs4803419 (15582C>T)	CC	TC	TT	0.07	0.19
		227 (0.87)	39 (0.12)	3 (0.01)		
CYP3A4	**rs35599367 (CYP3A4*22)**	GG	GA	AA	0.003	1
		317 (0.99)	2 (0.01)	0		
CYP3A5	**rs776746 (6986G>A)**	GG	GA	AA	0.82	0.44
		12 (0.04)	88 (0.28)	219 (0.69)		
NR1I3 (CAR)	**rs3003596**	AA	AG	GG	0.49	1
		78 (0.24)	159 (0.50)	82 (0.26)		
	rs2307424 (540C>T)	CC	CT	TT	0.08	0.42
		272 (0.85)	44 (0.14)	3 (0.01)		
NR1I2 (PXR)	**rs2472677 (63396C>T)**	CC	CT	TT	0.36	0.14
		124 (0.39)	160 (0.50)	35 (0.11)		
ABCC10	**rs2125739**	TT	CT	CC	0.23	0.27
		185 (0.58)	120 (0.38)	13 (0.04)		

Note: †number (proportion). Hom-Ref - homozygous for the functional allele; Het-LOF - heterozygous for the loss-of-function (LOF) allele; Hom-LOF - homozygous for the LOF allele; MAF – minor allele frequency; HWE - Hardy-Weinberg equilibrium.

Information for 319 children from CHAPAS-3 study (aside from rs2125739 – data on 318 children).

Table 6.5 Model estimated nevirapine clearance intrinsic and corresponding hepatic clearance, hepatic extraction, hepatic bioavailability and oral clearance by metaboliser status.

Metabolizer Status	CL_{int} [L/h]	CL_H [L/h]	E_H	F_H	CL_{oral} [L/h]
Fast	3.27	1.20	7.9%	91.1%	1.29
Intermediate	2.72	1.01	6.6%	93.4%	1.09
Slow	1.65	0.63	4.1%	95.9%	0.68
Very slow	1.04	0.40	2.6%	96.4%	0.43

Note: CL_{int} – clearance intrinsic; CL_H – clearance hepatic; E_H – hepatic extraction; F_H – hepatic component of bioavailability; CL_{oral} – oral clearance

The relationship between parameters and how they can be derived explained in the Appendix. Presented values relate to an average child of 14.5 kg, 4.1 years of age and corresponding pre-hepatic bioavailability of 93% and hepatic plasma flow of 15.35 (L/h).

6.9.3 Supplementary figures

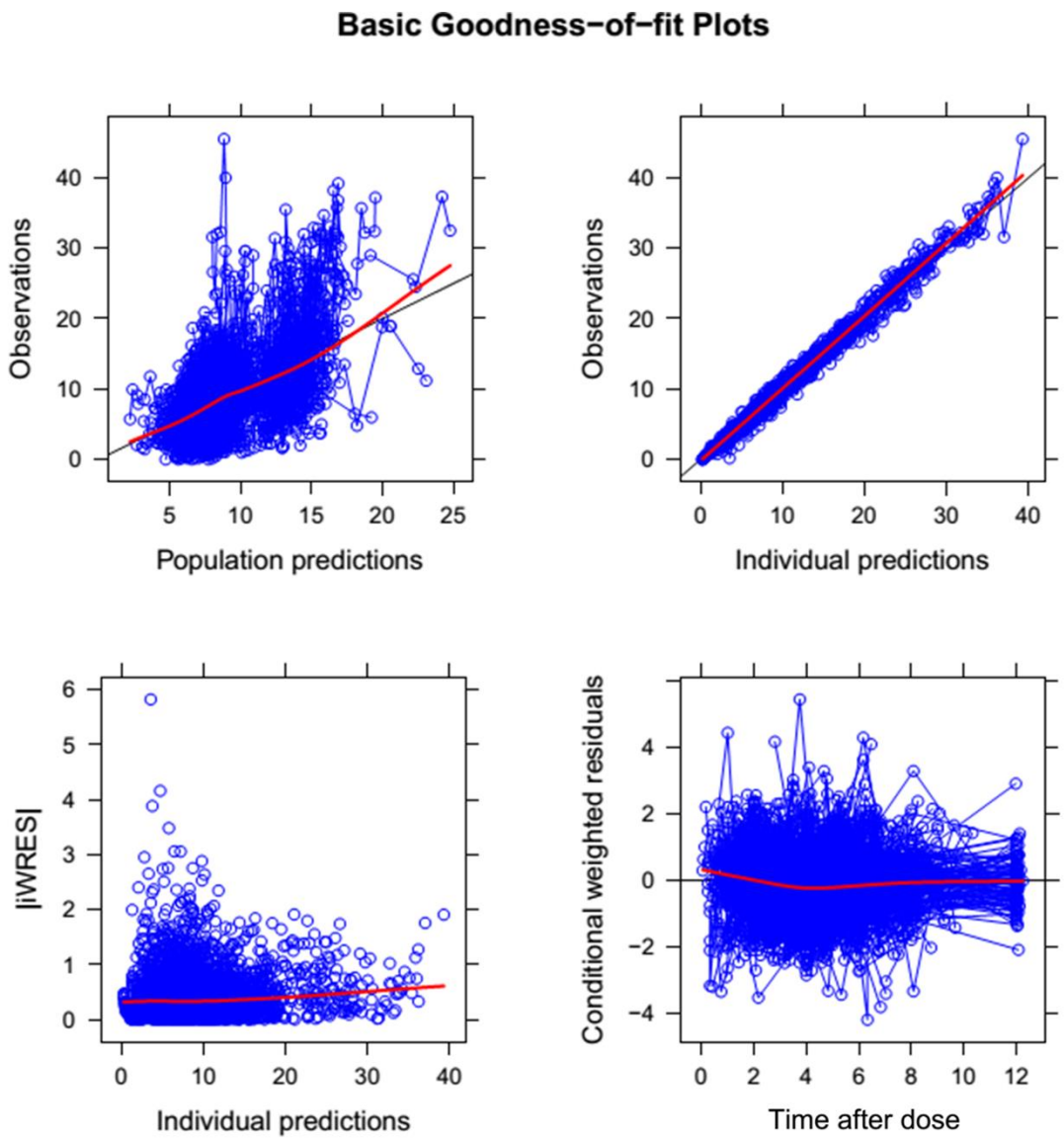

Figure 6.6 Goodness of fit plots for the final nevirapine population pharmacokinetic model

Note: Top left – observations vs population predictions (log scale); top right – observations vs individual predictions (log scale); bottom left – absolute values of individual weighted residuals vs individual predictions bottom right – conditional weighted residuals (CWRESI) vs time after dose.

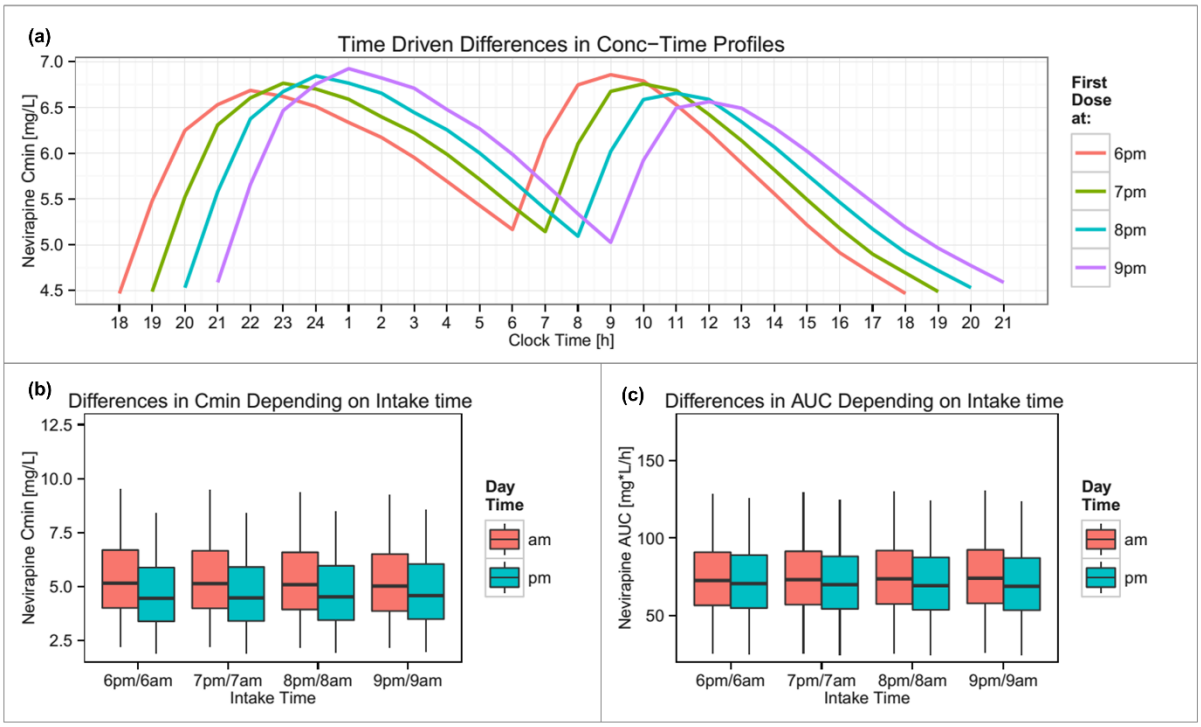

Figure 6.7 Results of simulations evaluating the effect of intake time on nevirapine exposures

Note: Plots show: (a) concentration-time curves for evaluated intake time scenarios; (b) differences between morning and evening C_{min} depending on intake time; (c) differences between morning and evening AUC depending on intake time.

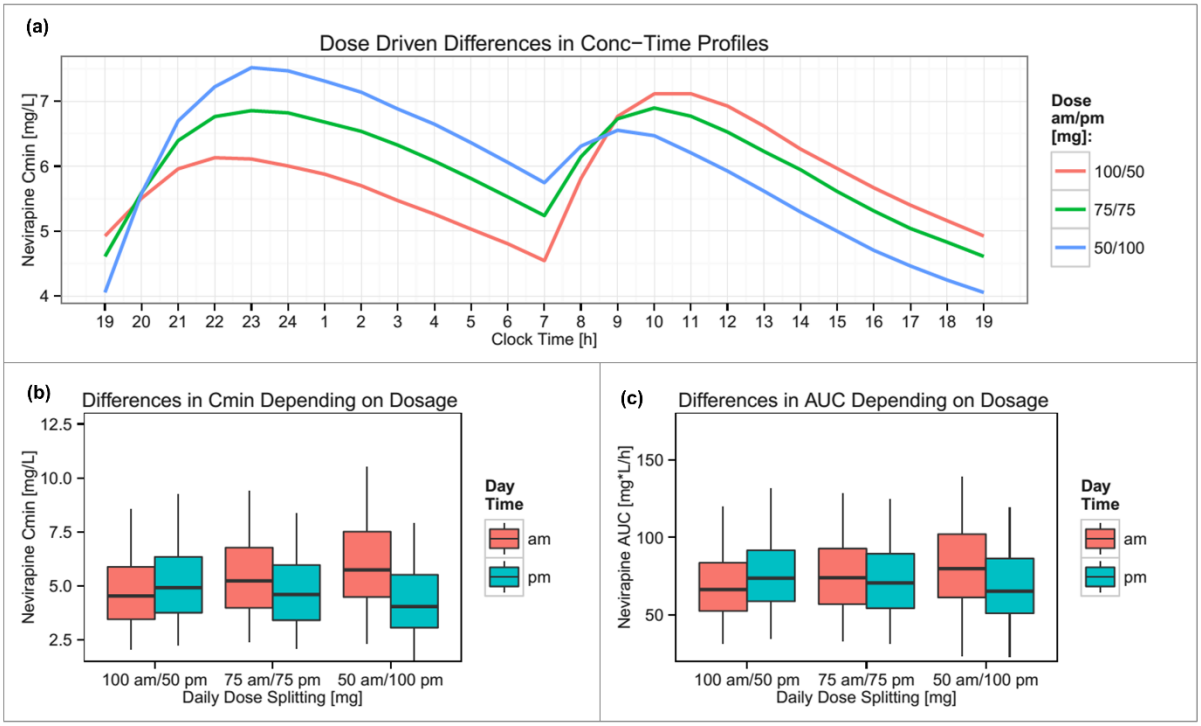

Figure 6.8 Nevirapine exposures obtained using different dose-splitting strategies

Note: Plots show: (a) concentration-time curves for the evaluated dosing scenarios; (b) differences between morning and evening C_{min} depending on dose-splitting strategy; (c) differences between morning and evening AUC depending on dose-splitting strategy.

6.9.4 NONMEM control stream

```
; Model descr: FINAL[LIVER][COMP-$DES][MIXTURE][DIURNAL][BIO-EFF;EXP]
; With code for AUC_TAU
$SIZES      MAXFCN=100000000 PD=-1000 LVR=-150 LTH=-200
$PROBLEM    PK NVP model
;-----------------------------------------------------------------------------------------------------------------------
$INPUT      ID TRIALNO=DROP CHAPAS EVENT WHAT=DROP WEEKNO OCC OCC_CL
            DAT1=DROP TIME CLOCK_START AMT DV MDV EVID BLQ NIGHT FLAG
            AGE WT HT SEX WAZ HAZ NRTI TB SITE DOSE_AM DOSE_PM
            DOSE_TOTAL TABLETS=DROP ART G516T T983C rs480349
            rs35599367 rs776746 rs3003596 rs2307424 rs2472677
            rs2125739 PGX3 MET MET2 MET3 MET4 TRAD_MED TRAD_MED_CODE
            TRAD_MED_TYPE=DROP COMMENTS=DROP
;------------------------------------------------------------------- ----------------------------------------------
$DATA      NVP_NONMEM_30Nov2015.csv IGNORE=@ IGNORE=(BLQ.EQ.1)

;-----------------------------------------------------------------------------------------------------------------------
$ABBREVIATED COMRES=2
$SUBROUTINE ADVAN13 TRANS1 TOL=8
;------------------------------------------------------------------- ----------------------------------------------
$MODEL      NCOMP=4 COMP=(DEPOT DEFDOSE) COMP=(LIVER)
          COMP=(CENTRAL DEFOBS) COMP=(AUC)
;-----------------------------------------------------------------------------------------------------------------------

;-----------------------------------------------------------------------------------------------------------------------
;; Initial estimates Theta and Omega
$THETA  (0,3.963850,20)    ; 1 FAST  TVCL [L/h]
$THETA  (0,25.15930,400)  ; 2 TVV2 [L]
$THETA  (0,0.819063,10)    ; 3 TVKA [1/h]
$THETA  (0,0.355995,2)     ; 4 ADD error
$THETA  (0,0.050466,1)     ; 5 PROP error []
$THETA  (0,0.492383,8)     ; 6 TVMTT [h]
$THETA  2 FIX                ; 7 NN []
$THETA  (0,1.609520,10)    ; 8 SPK ERROR
$THETA  (0,2.736170,10)    ; 9 INTERM
$THETA  (0,1.700710,10)    ; 10 SLOW
$THETA  (0,1.083350,10)    ; 11 U-SLOW
$THETA  (0,1.708910,10)    ; 12 NOT-OBS BOVBIO []
$THETA  (0,0.287272,1)     ; 13 AMP_CL
$THETA  (-24,-12.6037,24) ; 14 SHIFT_CL
$THETA  (0,0.592143,1)     ; 15 BIO_BIRTH []
$THETA  (0.05,0.4749,10)   ; 16 KBIO []
$OMEGA  0.037351           ; 1 BSVCL
$OMEGA  0  FIX             ; 2 BSVV2
$OMEGA  0  FIX             ; 3 BSVKA
$OMEGA  0.041468           ; 4 BSVBIO
$OMEGA  0  FIX             ; 5 BSVMTT
$OMEGA  BLOCK(1)
 0.024070                  ; 6 BOVBIO OCC1
$OMEGA  BLOCK(1) SAME
```

```
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1)
 0.199743                ; 14 BOVKA OCC1
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1)
 4.248400                ; 22 BOVMTT OCC1
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
;-------------------------------------------------------------------------------------------------------------------
$PK

;----- Allometric Scaling ----------------------------------------------------------------------------------------
  TVWT = 14.5  ; Median weight in kg
  ALLMCL=(WT/TVWT)**0.75
  ALLMV=WT/TVWT

;----- DEFINE VARIABILITY  ---------------------------------------------------------------------------------------
;----- BOV -------------------------------------------------------------------------------------------------------
 BOVBIO =ETA(6)  ;OCC=1 lag doses and pre-dose are treated as same occasion
 IF (OCC.GT.1.5.AND.OCC.LE.2.5)   BOVBIO = ETA(7)    ;OCC=2 (prevents NONMEM from crashing)
 IF (OCC.GT.2.5.AND.OCC.LE.3.5)   BOVBIO = ETA(8)    ;OCC=3
 IF (OCC.GT.3.5.AND.OCC.LE.4.5)   BOVBIO = ETA(9)    ;OCC=4
 IF (OCC.GT.4.5.AND.OCC.LE.5.5)   BOVBIO = ETA(10)  ;OCC=5
 IF (OCC.GT.5.5.AND.OCC.LE.6.5)   BOVBIO = ETA(11)  ;OCC=6
 IF (OCC.GT.6.5.AND.OCC.LE.7.5)   BOVBIO = ETA(12)  ;OCC=7
 IF (OCC.GT.7.5.AND.OCC.LE.8.5)   BOVBIO = ETA(13)  ;OCC=8

; CORRECTION FACTOR FOR NOT OBSERVED INTAKE - EFFECT ON BIO
 NOT_OBS = THETA(12)
 IF (OCC.EQ.1.OR.CHAPAS.EQ.3) BOVBIO= NOT_OBS *BOVBIO

 BOVKA =ETA(14)  ;OCC=1 lag doses and pre-dose are treated as same occasion
 IF (OCC.GT.1.5.AND.OCC.LE.2.5)   BOVKA = ETA(15)  ;OCC=2
 IF (OCC.GT.2.5.AND.OCC.LE.3.5)   BOVKA = ETA(16)  ;OCC=3
 IF (OCC.GT.3.5.AND.OCC.LE.4.5)   BOVKA = ETA(17)  ;OCC=4
```

```
 IF (OCC.GT.4.5.AND.OCC.LE.5.5)   BOVKA = ETA(18)  ;OCC=5
 IF (OCC.GT.5.5.AND.OCC.LE.6.5)   BOVKA = ETA(19)  ;OCC=6
 IF (OCC.GT.6.5.AND.OCC.LE.7.5)   BOVKA = ETA(20)  ;OCC=7
 IF (OCC.GT.7.5.AND.OCC.LE.8.5)   BOVKA = ETA(21)  ;OCC=8

 BOVMTT =ETA(22)  ;OCC=1 lag doses and pre-dose are treated as same occasion
 IF (OCC.GT.1.5.AND.OCC.LE.2.5)   BOVMTT = ETA(23)   ;OCC=2
 IF (OCC.GT.2.5.AND.OCC.LE.3.5)   BOVMTT = ETA(24)   ;OCC=3
 IF (OCC.GT.3.5.AND.OCC.LE.4.5)   BOVMTT = ETA(25)   ;OCC=4
 IF (OCC.GT.4.5.AND.OCC.LE.5.5)   BOVMTT = ETA(26)   ;OCC=5
 IF (OCC.GT.5.5.AND.OCC.LE.6.5)   BOVMTT = ETA(27)   ;OCC=6
 IF (OCC.GT.6.5.AND.OCC.LE.7.5)   BOVMTT = ETA(28)   ;OCC=7
 IF (OCC.GT.7.5.AND.OCC.LE.8.5)   BOVMTT = ETA(29)   ;OCC=8

;------------------  BSV ---------------------------------------------------------------------------------------------
  BSVCL  =ETA(1)
  BSVV2  =ETA(2)
  BSVKA  =ETA(3)
  BSVBIO =ETA(4)
  BSVMTT =ETA(5)

;----- DEFINE POPULATION PARAMETERS ----------------------------------------------------------------------------
;----  CL BASED ON MIXTURE MODEL ------------------------------------------------------------------------------
EST=MIXEST

MET_EST=MET                                        ; patients with available genotype

; for patients with missing genotype:
IF(MET.EQ.99.AND.MIXNUM.EQ.1) MET_EST=1 ; EXT - from mixture model
IF(MET.EQ.99.AND.MIXNUM.EQ.2) MET_EST=2 ; INTERMEDIATE - from mixture model
IF(MET.EQ.99.AND.MIXNUM.EQ.3) MET_EST=3 ; SLOW - from mixture model
IF(MET.EQ.99.AND.MIXNUM.EQ.4) MET_EST=4 ; ULTRA-SLOW - from mixture model

IF(MET_EST.EQ.1) CLMET=THETA(1)
IF(MET_EST.EQ.2) CLMET=THETA(9)
IF(MET_EST.EQ.3) CLMET=THETA(10)
IF(MET_EST.EQ.4) CLMET=THETA(11)

;------------------------------------------------------------------- ----------------------------------------------

  TVCL  = CLMET *ALLMCL   ; typical value of CL
  TVV2  = THETA(2)*ALLMV ; typical value of V
  TVKA  = THETA(3)            ; typical value of KA
  TVMTT = THETA(6)            ; typical value of MTT
  TVNN  = THETA(7)            ; typical value of NN
  TVBIO = 1                   ; pre-hepatic oral bio fix to 1

;----- DEFINE INDIVIDUAL PARAMETERS ----------------------------------------------------------------------------
 CL   = TVCL  *EXP(BSVCL)              ; individual value of CL
 V2   = TVV2  *EXP(BSVV2)              ; individual value of V
 KA   = TVKA  *EXP(BSVKA+BOVKA)  ; individual value of KA
```

```
 MTT  = TVMTT *EXP(BSVMTT+BOVMTT); individual value of MTT
 NN   = TVNN                       ; individual value of NN

;------------  AGE effect on BIO  ------------------------------------------------------------------------------------
  BIO_BIRTH =THETA(15)                                  ; bio at birth
  KBIO      =THETA(16)                                  ; age effect constant

; AGE EFF ON F1 AS INVERSE EXP WITH INTERCEPT
  AGEBIO = 1 - ((1-BIO_BIRTH)*(EXP(-(AGE*KBIO))))      ; age in years from birth

  BIO = TVBIO *EXP(BSVBIO+BOVBIO) *AGEBIO              ; individual value pre-hepatic bio

;------------------- ASSUMPTIONS FOR DIURNAL ----------------------------------------------------------------------
  AMP_CL   = THETA(13)                  ; amplitude of circadian rhythm
  SHIFT_CL = THETA(14)                  ; shift of actophase (max) from midday

;------------------- ASSUMPTIONS FOR LIVER FIRST PASS -------------------------------------------------------------
 CLINT=CL
 FU = 0.4                               ; fraction unbound in plasma
 QH = 50 *(WT/70)**0.75                 ; hepatic plasma flow - adult = 50L/h
 VH = 1 *(WT/70)                        ; volume of liver

;------------------- TRANSFER RATE CONSTANTS ----------------------------------------------------------------------
 K32 = QH/V2                            ; from central back to liver

 ; rates from liver to central and extraction rate const defined in $DES

; RESET code for Cmax Tmax
IF (NEWIND/=2.OR.EVID>=3) THEN
        COM(1)=0
        COM(2)=0
        TIMEDOSE = TIME
        AMOUNTDOSE = AMT
        AUC_START  = 0
        AUC_STOP   = 0
        AUC_TAU    = 0
ENDIF
 ;----------------------  TRANSIT  ------------------------------------------------------------------------------------

F1=0 ; I need to set bioavailability in compartment 1 to 0


KTR = (NN+1)/MTT

IF (NEWIND/=2.OR.EVID>=3) THEN ; new individual, or reset event
   ; The values read here will be stored in TDOS and PD in this very PK call
        TNXD=TIME ; Time of the dose
        PNXD=AMT ; Amount. If it's zero, the DE is deactivated.
ENDIF

TDOS=TNXD ; This will either save here the temporary values if it's a new individual...
```

```
PD=PNXD ; ...or the values which were read one record ahead during the execution of the previous record.

IF(AMT.GT.0) THEN ; This reads one record ahead and stores the data to be used when running the following record
        TNXD=TIME
        PNXD=AMT
ENDIF

LNGAM = NN*LOG(NN)-NN+LOG(NN*(1+4*NN*(1+2*NN)))/6+0.572364942
; approximation of log of gamma(n), 0.572364942 is LOG(PI)/2
; To speed up the computation, I calculate here all the non-time-varying quantities used in $DES
PIZZA=LOG(BIO*PD*KTR+0.00001)-LNGAM
; without +0.00001, it won't work with ETAs in bioavailability

;---------------- Initialisation for DES solver  ----------------------------------------------------------------------------
; SS option causes model to crash due to some low concentrations
; Instead initialise the compartments with calculated SS Cmin
; Based on individual parameter values
; Followed with additional 5 days of dosing to make sure patient is at steady state

 TAU_EQ=MTT+1/KA
 KA_EQ=1/TAU_EQ                                  ; because we're using TRANSIT absoprtion

;-------CALCULATE TEMP VALUE OF K WITH DISREGARD TO CIRCADIAN RHYTHM----
 EH_TEMP = (FU * CLINT) / (QH +(FU *CLINT))  ; hepatic extraction ratio
 VTOT    = VH+V2                                 ; approx as sum of liver and central
 K_TEMP  =(QH*EH_TEMP)/VTOT                      ; elimination rate constant

;-- dosing is every 12h, here use hf of the total dose (just approx)
  TAU = 12 ; interdose interval

  BASELINE = ((BIO*(1-EH_TEMP) * DOSE_TOTAL/2 * KA_EQ) / (VTOT * (KA_EQ - K_TEMP)))  * ( (1 / (1- EXP(-K_TEMP * TAU))) - (1 /( 1- EXP(-KA_EQ*TAU))))

  A_0(1)= 0.0001                ; initialise absorption CMT
  A_0(2)= BASELINE *VH          ; initialise liver CMT
  A_0(3)= BASELINE *V2          ; initialise central CMT

$DES
;------------------- First pass WITH DIURNAL VARIATION ----------------------------------------------------------------
TIME_CLOCK=T+CLOCK_START ; calculate time progress in relation to first event (actual clock time)

; diurnal oscillation with time expressed as shift from midnight
DIURNAL_EF=EXP(AMP_CL*COS((TIME_CLOCK-SHIFT_CL)*6.283/24)) ; 2*PI=6.283
CL_DIURNAL=CLINT *DIURNAL_EF

 EH =(FU * CL_DIURNAL) / (QH +(FU *CL_DIURNAL))     ; hepatic extraction ratio
 FH = 1 - EH                                  ; BIO after first pass metabolism
 CLH= QH *EH                                  ; part metabolised
 K   =(QH*EH)/VH                              ; Metabolic rate constant from liver - ELIMINATION
```

```
 K23 =(QH*FH)/VH                    ; part that goes to cent CMT

;-------------------------------------------------------------------------------------------------------------------------------
TEMPO=T-TDOS ; this is time after dose, it should always be >= 0
KTT=0

DADT(1)=0

IF(PD.GT.0.AND.TEMPO.GT.0) THEN ; This happens only id PD>0, so only if a dose has been detected
        KTT=KTR*(TEMPO)
        DADT(1)=EXP(PIZZA+NN*LOG(KTT)-KTT)-KA*A(1)
ENDIF

DADT(2)= KA*A(1)- K*A(2)- K23*A(2)+K32*A(3)
DADT(3)= K23*A(2)-K32*A(3)
DADT(4)= A(3)/V2                   ; integral of conc for AUC 0-tau


; For Cmax Tmax
TIMEAFTERDOSE=T-TIMEDOSE
CP = A(3)/V2                       ; plasma concentration
IF (CP.GE.COM(1)) THEN
        COM(1) = CP                ; CMAX
        COM(2) = TIMEAFTERDOSE     ; time of CMAX
ENDIF

;------------------------------------ MIXTURE MODEL  ---------------------------------------------------------------
$MIX      NSPOP=4
; proportions set to ones observed in the study
P(1) = 107/323                     ; prop of EXT
P(2) = 144/323                     ; prop of INTER
P(3) = 70/323                      ; prop of SLOW
P(4) = 2/323                       ; prop of U-SLOW
;-------------------------------------------------------------------------------------------------------------------------------
$ERROR

 IPRED = A(3)/V2                   ; Individual prediction in central CMT
 IRES  = DV - IPRED                ; Individual residual

 WA    = THETA(4)                  ; Additive error
 WP    = IPRED * THETA(5)          ; Prop error
 W     = SQRT(WA**2 + WP**2)       ; Weighting factor for residuals
 IF(W.LE.0.0001) W = 0.0001        ; Protection against division with 0

 IF(CHAPAS.GT.1.5) W = W*(THETA(8))     ; Additional error term for sparse data

 IWRES = IRES / W
 Y     = IPRED + W*EPS(1)   ; Model prediction of observed PK value with additive + proportional error

AA1=A(1)            ; abs CMT
AA2=A(2)            ; LIVER CMT
```

```
 AA3=A(3)              ; central CMT
CMAX = COM(1)     ; CMAX
TMAX = COM(2)      ; time of CMAX

IF(AMT.GT.0) THEN
          TIMEDOSE = TIME
          AMOUNTDOSE = AMT
; Reset CMAX code when a new dose is given
          COM(1)=0
          COM(2)=0
ENDIF

  TAD=TIME-TIMEDOSE
  TVPC=TIME-132            ; approx for plotting VPC
  ; am_intake is 132h post start of dose OCC (5x pre-doses starting with pm_intake)
  CL_ORAL =FU*CL           ; approx from Rowland and Tozer

  VAR_AUC= (BSVBIO + BOVBIO) - (BSVCL)   ; for diagnostic plots
  VAR_BIO= BSVBIO + BOVBIO               ; for diagnostic plots

;---- CALCULATE integral AUC FROM AMT IN 4TH COMP ------------------------------------------------------------
; due to diurnal variation AUC differs between daytime and nightime
; needs to be calculated as exact integer of concentrations within 12h dosing interval
 IF(EVENT.EQ.1)   AUC_START=A(4)          ; flag for dose
 IF(EVENT.EQ.120) AUC_STOP =A(4)          ; flag for 12h post dose
 AUC_TAU= AUC_STOP - AUC_START            ; AUC 0-12h

  IF (ICALL==4.AND.Y.LE.0.1) Y=0.05       ; prevents negative simulated values for VPC
;-------------------------------------------------------------------------------------------------------------------------------------------
$SIGMA  1  FIX  ; Scaled RUV variance - IT'S A VARIANCE SO FIXED TO "0" AND ALL ERROR IS GOING TO
THE THETAS AND IT MAKES A SD
$ESTIMATION MSFO=msf555 MAXEVAL=0 PRINT=1 METHOD=1 INTER MCETA=1000
          RANMETHOD=4P ETASTYPE=1 NONINFETA=1 NOABORT NSIG=3 SIGL=9
          ATOL=6 ; calculation method
$ESTIMATION MSFO=msf555 MAXEVAL=9999 PRINT=1 METHOD=1 INTER MCETA=5
          RANMETHOD=4P ETASTYPE=1 NONINFETA=1 NOABORT NSIG=3 SIGL=9
          ATOL=6 ; calculation method

$COVARIANCE PRINT=E ; standard error of estimate is calculated

$TABLE      WRESCHOL ID DV OCC OCC_CL TAD TVPC TIME PRED IPRED
          DOSE_TOTAL AA1 AA2 AA3 IWRES WRES CWRES CWRESI OBJI
          NOPRINT ONEHEADER FILE=sdtab555
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          AUC_INF VAR_AUC VAR_BIO BSVCL BSVV2 BSVKA BSVBIO BOVBIO
          BOVKA BOVMTT WA WP NOPRINT NOAPPEND ONEHEADER
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        rs2307424 rs2472677 rs2125739 PGX3 MET MET2 MET3 MET4 BLQ
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        BOVBIO NOT_OBS BOVKA BOVMTT WA WP AUC_INF AUC_START
        AUC_STOP AUC_TAU VAR_AUC VAR_BIO CMAX TMAX AGE AMT HT WT
        WAZ HAZ SEX DOSE_TOTAL DOSE_AM DOSE_PM NRTI ART G516T
        T983C rs480349 rs35599367 rs776746 rs3003596 rs2307424
        rs2472677 rs2125739 PGX3 MET MET2 MET3 MET4 MET_EST BLQ
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CHAPTER 7: DETERMINANTS OF VIROLOGICAL OUTCOME AND ADVERSE EVENTS IN AFRICAN CHILDREN TREATED WITH PAEDIATRIC NEVIRAPINE FIXED-DOSE-COMBINATION TABLETS.

7.1 Abstract

Background

Nevirapine is the only non-nucleoside reverse transcriptase inhibitor currently available as a paediatric fixed-dose combination tablet and is widely used in African children. Nonetheless, the number of investigations into pharmacokinetic determinants of virological suppression in African children is limited and the predictive power of the current therapeutic range was never evaluated in this population, thereby limiting treatment optimisation.

Methods

We analysed data from 322 African children (aged 0.3–13 years) treated with nevirapine, lamivudine, and either abacavir, stavudine, or zidovudine, and followed up to 144 weeks. Nevirapine trough concentration (C_{min}) and other factors were tested for associations with viral load (VL)>100 copies/mL and transaminase increases >grade 1 using proportional hazard and logistic models in 219 initially antiretroviral treatment (ART)-naïve children.

Results

Pre-ART VL, adherence, and nevirapine C_{min} were associated with VL non-suppression (hazard-ratio [HR]=2.08 [95% CI: 1.50-2.90, p<0.001] for 10-fold higher pre-ART VL, HR=0.78 [95% CI: 0.68–0.90, p<0.001] for 10% improvement in adherence and HR=0.94 [95% CI: 0.90-0.99, p=0.014] for a 1mg/L increase in nevirapine C_{min}). There were additional effects of pre-ART CD4% and clinical site. The risk of virological non-suppression decreased with increasing nevirapine C_{min} and there was no clear C_{min} threshold predictive of virological non-suppression. Transient transaminase elevations >grade 1 were associated with high C_{min} (>12.4 mg/L), HR=5.18 (95%CI 1.95–13.80, p<0.001).

Conclusions

Treatment initiation at lower pre-ART VL and higher pre-ART CD4%, increased adherence, and maintaining average C_{min} higher than current target could improve virological suppression of African children treated with nevirapine without increasing toxicity.

7.2 Introduction

Fixed-dose combination (FDC) formulations have considerably improved access to antiretroviral treatment (ART) through decreased cost and improved feasibility especially in Sub-Saharan Africa.[33] Currently available paediatric dispersible FDCs are limited to combinations of a non-nucleoside reverse transcriptase inhibitor (NNRTI), nevirapine, with two nucleoside-reverse transcriptase inhibitors (NRTIs), and are widely used in children in low-income countries.[111]

Nevirapine pharmacokinetics (PK) exhibits high variability, attributed in part to single nucleotide polymorphisms (SNPs) of cytochrome *P450 2B6* (*CYP2B6)*, which encode an important metabolic pathway for this drug.[43,72,431] In adults, low nevirapine concentrations have been associated with increased risk of virological failure[340–342] and high exposures with increased risk of skin rashes[336,346,358] and hepatotoxicity.[341,352] Based on the concentration-response relationship, a therapeutic range of 3–8 mg/L has been suggested for nevirapine therapeutic drug monitoring (TDM).[95] However, several studies failed to confirm these associations[72,104,105] and low incidences of nevirapine-related adverse events (AEs) have been reported in low-income settings[106] and in African children.[107,108] Despite widespread use, few studies have investigated the pharmacokinetic determinants of efficacy of nevirapine-based regimens in children. The predictive power of the suggested targets has also never been thoroughly investigated in black Africans or in children. Whether these pharmacokinetic targets should be universally applied across populations was recently questioned.[72]

Our aim was therefore to investigate associations between nevirapine trough concentrations (C_{min}) and long-term virological outcomes and AEs in African children, and establish if any other factors predict treatment outcome after adjusting for drug exposures, allowing treatment optimisation.

7.3 Methods

7.3.1 Population and study design

The CHAPAS-3 trial enrolled HIV-infected ART-naïve and ART-experienced (>2 years ART with viral load (VL) <50 copies/ml at screening) children aged 0.3–13 years from four sites in Uganda and Zambia,[382] treated following WHO 2010 guidelines[11] with an NNRTI (nevirapine or efavirenz) and 2 NRTIs (lamivudine plus randomised abacavir, stavudine, or zidovudine). Nevirapine was co-formulated with companion NRTIs in paediatric FDCs provided by Cipla (India).[382] Children on nevirapine were switched to efavirenz-based ART if aged >3 years and diagnosed with tuberculosis or experienced nevirapine-

related AEs, or to boosted protease inhibitor-based (PI) ART if <3 years with these events or for clinical or immunological failure (or if efavirenz-intolerant). Samples for PK analysis (described previously)[431] were taken at week 6, 36, and every 24 weeks thereafter. VL was assayed retrospectively on stored plasma taken at enrolment and weeks 48, 96 and 132 or 144.

7.3.2 Statistical analysis

A previously developed model describing the steady-state population-PK of nevirapine[431] was used to derive empirical Bayesian estimates for each child at each PK visit for: CL (clearance), C_{min} (evening trough concentration), C_{max} (maximum concentration), and AUC_{0-24} (area under the curve). Due to diurnal variability in clearance[431], this analysis included measurements relating to daytime exposures only.

The primary efficacy outcome was VL > 100 copies/mL (the limit of detection as many samples had to be diluted due to low volumes). For descriptive analysis, response was categorized as: suppressed (<100 copies/mL within 48 weeks of treatment initiation, maintained throughout follow-up), slow suppression (<100 copies/mL achieved after 48 weeks but maintained throughout subsequent follow-up), rebounded (<100 copies/mL within 48 weeks but VL>100 copies/mL at single or multiple visits thereafter) and never suppressed (VL never <100 copies/mL). ART-experienced children (all VL<50 copies/ml at enrolment) were analysed separately from ART-naïve children initiating treatment at enrolment. Since multiple PK exposures were available for each child, geometric means of PK parameters across follow-up within each individual, and inter-individual variability (expressed as coefficient of variation [CV][432]) for C_{min} and AUC_{0-24} were compared between groups using Kruskal-Wallis and rank-sum tests. Categorical factors were compared between groups using Fisher's exact test.

The effect of nevirapine C_{min} on the risk of virological non-suppression (>100 copies/ml) in the subset of ART-naïve children only was estimated using Cox proportional hazards regression models (Andersen-Gill repeated outcomes framework) with Efron approximation in R (survival package).[380,402–404] VLs were matched with the model estimated C_{min} from the closest sampling visit preceding each VL. Each time interval ran from the preceding to the current VL (classified as suppressed vs non-suppressed "event") and the matched C_{min} was applied to the whole time interval. Non-linearity in the effect of C_{min} was explored visually using smoothed splines, and tested using fractional polynomials (Stata 14.0 mfp cox).[401] As Cox regression does not provide estimates of the absolute probability of suppression, we estimated this using a mixed-effects repeated measures logistic model (Stata 14.0 mfp logistic).[401] The best-fitting dichotomous threshold for nevirapine C_{min} in the Cox model was

identified by profile likelihood as described previously for efavirenz.[433] Following the same method, we conducted simulations introducing unexplained residual variability on predicted concentrations from the population-PK model (additive error 0.32 mg/L, proportional error 5.26%)[431] to derive 95% confidence intervals (CI) for this threshold (2.5th and 97.5th percentile of most predictive cut-offs from 500 simulations). The sensitivity, specificity, accuracy, and positive and negative predictive values of the identified threshold for VL suppression were compared to those of the 10th, 25th, and 50th percentiles of estimated nevirapine C_{min} in this study, and cut-offs proposed in the literature.[95,340,341]

Finally, we used backwards elimination (exit p=0.05, retaining all levels of categorical factors where any were p<0.05) to consider the additional independent effects on non-suppression of factors with associations (p<0.2) in univariate models. Categorical covariates included NRTI-backbone (abacavir, zidovudine, or stavudine), sex, clinical site, exposure to ART in children and/or mothers in prevention of mother to child transmission (pMTCT [regimens listed in footnotes to Tables 7.1 and 7.2]), metaboliser status (MET) based on *CYP2B6* 516G>T|983T>C single nucleotide polymorphisms (SNP) (extensive metabolisers [EM] - 516GG|983TT; intermediate metabolisers [IM] - 516GG|983TC or 516GT|983TT, slow metabolisers [SM] - 516TT|983TT or 516GT|983TC; ultra-slow metabolisers [USM] - 516GG|983CC),[431] mother as primary carer, self-reported missing any ART doses in previous 4 weeks. Continuous variables included baseline (pre-ART) viral load (bVL) and CD4% (bCD4%, truncated at 50% to avoid undue influence of outliers), and current age, weight-for-age Z-score (WAZ),[416] height-for-age Z-score (HAZ),[416] and adherence (percentage of doses taken based on MEMS-cap container openings in the interval between previous and current VL [truncated to a lower limit of 0.5]). The only factor with incomplete information was adherence; when no data was available for current interval, the preceding interval's value was carried forward. If no prior MEMS data were available (n=21) we imputed the median value for all ART-naïve individuals. Non-linear effects in continuous variables were explored as described above for C_{min}. Interactions between factors included in the final model were investigated and included if p<0.05.

7.3.3 Adverse events (AEs)

AEs considered to be nevirapine-related were: hypersensitivity reactions (HSR, including Stevens-Johnson Syndrome [SJS]), raised liver enzymes (aspartate or alanine transaminase [AST or ALT] >grade 2, i.e. >5x ULN), and acute hepatitis. The characteristics of children developing AEs were compared to others using Fisher's exact or rank-sum tests. AST and ALT were measured at enrolment and weeks 6, 12, 24, and 24-weekly throughout the study and were matched with nevirapine C_{min} as described for VL. The association between NVP C_{min} (and all covariates above plus pre-ART >grade 1 transaminase

elevation) and the risk of developing >grade 1 AST or ALT, i.e. >2.5x ULN (composite endpoint) were estimated in the ART-naïve group as for virological non-suppression. Additionally, in the same group, the change from baseline in transaminase levels at weeks 6, 48, and 96 was compared using Wilcoxon signed rank test separately for children with C_{min} below and above the threshold identified most predictive of transaminase >1 grade elevations by likelihood profiling as explained above. Probabilities of AEs were similarly estimated using mixed-effects logistic regression.

7.4 Results

7.4.1 Patient characteristics

Of 478 children in CHAPAS-3, 338 received nevirapine (99 ART-experienced) combined with a 2-NRTI backbone, and contributed 3340 PK samples (1566 dosing intervals, 1-6 per individual) and 718 VLs post enrolment (1-3 per individual). Sixteen individuals (all ART-naïve) changed ART: 9 to efavirenz (when tuberculosis was diagnosed) and 7 to protease-inhibitors (3 for AEs, 4 for clinical failure [2 in year 2 and 2 in year 3]). The demographic characteristics and model-estimated PK parameters for children included in this analysis are shown in Tables 7.1 and 7.2 by virological response group.

Amongst ART-naïve children, 151 (68%) achieved and maintained VL <100 copies/mL, 125 (56%) by week 48. Those who took longer to suppress had almost three times higher pre-ART VL and lower CD4-counts. Amongst ART-naïve participants who suppressed by week 48, 27 rebounded and the majority of these children re-suppressed during follow-up. Pre-ART CD4% in these rebounders was lower than other groups, and median pre-ART VL between that for the suppressed group and for those taking longer to suppress or never suppressed. The remaining 45 ART-naive children (20%) never suppressed VL to <100 copies/mL, but only 4 showed clinical evidence of treatment failure. Individuals who never suppressed had significantly higher pre-ART VL than children who suppressed by week 48, lower adherence than the other ART-naïve children (82% vs 93%, ranksum p=0.005) and lower nevirapine PK exposures (p<0.05) with higher levels of intra-individual variability (p=0.02), possibly indicating erratic adherence patterns.

Table 7.1 Demographic characteristics and model-derived PK parameters in different suppression groups of children in CHAPAS-3 treated with nevirapine (part 1)

Treatment status at enrolment		**Naïve**				**p***	**Experienced**	**p****
Suppression Group		**Suppressed**	**Slow Suppress**	**Rebound**	**Never Suppressed**			
Number of patients (row %)		125 (56%)	26 (12%)	27 (12%)	45 (20%)		99	
Baseline	**Age [years]**	2.0 (0.7-4.9)	2.2 (0.9-3.8)	1.9 (1.1-8.5)	1.6 (0.7-3.5)	0.114	6.1 (5.1-10.6)	<0.001
	Weight [kg]	9.8 (6.4-16.2)	10.4 (6.1-15.4)	9.8 (6.6-19.9)	8.6 (5.8-13.8)	0.016	19.0 (15.3-24.5)	<0.001
	CD4% [%]	21.0 (7.4-42.6)	19.4 (7.6-44.7)	16.5 (6.6-39.4)	19.0 (9.0-38.5)	0.054	34.5 (21.9-48.0)	<0.001
	CD4 [cells/ mm^3]	1137 (397-3289)	832 (209-1699)	732 (277-2061)	1025 (353-2453)	0.016	1259 (627-2237)	<0.001
	Viral Load [copies/ mL]	245’250 (6’506-1.7 mil)	663’047 (149’283-12.7 mil)	325’980 (43’144-3.8 mil)	555’130 (132’393-5.4 mil)	<0.001	< 50	<0.001
	Sex (M/F)	68/57	15/11	8/19	28/17	0.012	49/50	0.017
	pMTCT (Y/N)	22/103	5/21	5/22	6/39	0.895	8/91	0.214
WHO Stage	**1**	19	4	2	4	0.659	22	0.532
	2	46	8	10	22		23	
	3	53	12	13	14		38	
	4	7	2	14	5		16	
Metabolic Subgroup	**EM**	41	11	11	12	0.555	32	0.611
	IM	59	8	11	25		41	
	SM	24	7	5	7		26	
	USM	1	0	0	1		0	

Note: Numbers are number or median (5th and 95th percentile). M – male, F – female. pMTCT – exposure to any ART in children or mothers in prevention of mother to child transmission (in ART-naïve group 29 children and 28 mothers had any exposure to ART in pMTCT: 20 children were exposed to NVP [14 sdNVP and 6 NVP >2 days] and 14 children to zidovudine [5 in addition to NVP], 25 mothers were exposed to NVP [24 sdNVP and 1 NVP >2 days] and 5 mothers to zidovudine [3 in addition to NVP]; in ART-experienced group 4 children and 8 mothers had any exposure to ART in pMTCT: all children were exposed to NVP [all sdNVP] and none to zidovudine, all mothers were exposed to NVP [all sdNVP] and none to zidovudine). EM (CYP2B6 extensive metabolisers) - 516GG|983TT; IM (CYPT2B6 intermediate metabolisers) - 516GG|983TC or 516GT|983TT, SM (CYP2B6 slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (CYP2B6 ultra-slow metabolisers) - 516GG|983CC.

*Kruskal Wallis or Fisher’s Exact test comparing 4 groups of originally treatment-naïve children only. **Kruskal Wallis or Fisher’s Exact test comparing 5 groups including children who were treatment-experienced at enrolment.

Table 7.2 Demographic characteristics and model-derived PK parameters in different suppression groups of children in CHAPAS-3 treated with nevirapine (part 2 - continuation)

Treatment status at enrolment		Naïve				p*	Experienced	p**
Suppression Group		Suppressed	Slow Suppress	Rebound	Never Suppressed			
NRTI	d4T	40	11	11	16	0.424	27	0.070
	ZDV	36	4	8	17		42	
	ABC	49	11	8	12		30	
PK	C_{min} [mg/L]	5.43 (2.48-14.66)	6.68 (1.79-15.21)	5.21 (1.38-12.33)	4.76 (1.45-9.08)	0.043	6.57 (3.44-16.32)	<0.001
	AUC [mg*L/h]	80.8 (36.8-196.8)	94.1 (25.2-202.7)	71.9 (17.1-167.6)	66.5 (18.2-128.6)	0.022	92.1 (50.1-216.9)	<0.001
	C_{max} [mg/L]	7.84 (3.61-17.84)	9.26 (2.66-18.27)	6.87 (1.68-15.39)	6.34 (1.76-12.02)	0.015	8.90 (4.96-19.58)	<0.001
	CL [L/h]	0.91 (0.44-1.42)	0.99 (0.39-1.41)	0.91 (0.55-1.47)	0.85 (0.52-1.26)	0.68	1.20 (0.59-1.90)	<0.001
Adherence	MEMS Score [%]	93.0 (63.1-98.7)	94.9 (76.3-98.9)	92.8 (58.0-97.2)	82.0 (50.0-97.4)	0.012	90.2 (59.8-99.2)	0.017
	CV C_{min} [%]†	31.0 (9.7-237.4)	28.5 (7.4-307.1)	31.0 (10.2-246.6)	45.0 (11.0-395.0)	0.022	21.0 (8.0-103.2)	<0.001
	CV AUC [%]†	32.0 (9.4-291.7)	30.5 (11.5-391.7)	37.0 (8.0-308.0)	65.5 (12.0-420.0)	0.022	22.0 (8.9-124.4)	<0.001

Note: Numbers are number or median (5^{th} and 95^{th} percentile). For time-varying PK exposures and adherence, medians are of the geometric mean per child over all follow-up. Presented PK parameters relate to exposures following the morning dose. Included patients received nevirapine and had at least one PK visit. NRTI – nucleoside reverse transcriptase inhibitor, d4T – stavudine, ZDV – zidovudine, ABC – abacavir, CV – coefficient of variation[432]

*Kruskal Wallis or Fisher's Exact test comparing 4 groups of originally treatment-naïve children only. **Kruskal Wallis or Fisher's Exact test comparing 5 groups including children who were treatment-experienced at enrolment. †Only children with >1 sampling visit.

ART-experienced children were much older, with VL <50 copies/mL and higher CD4% at enrolment. The average C_{min} and AUC in this group were also higher than most ART-naïve children (p<0.001). Despite comparable MEMS-adherence-scores, ART-experienced children had significantly lower intra-individual variability in nevirapine PK measures than ART-naïve children (p<0.001), which might suggest more consistent adherence. Virological outcomes remained excellent: 88 (89%) remained suppressed <100 copies/mL throughout the study, 10 (10%) had a virological rebound and only 1 child had all VL measurements >100 copies/mL.

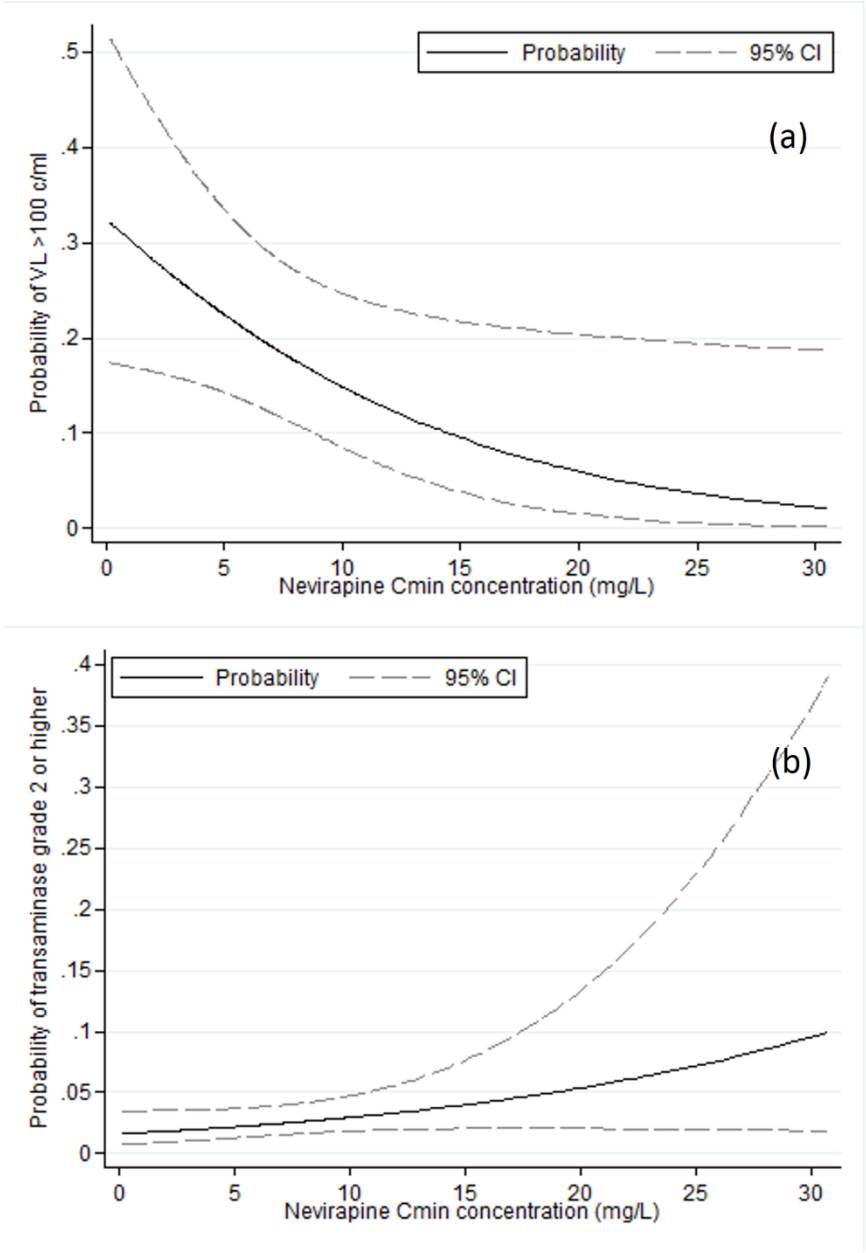
7.4.2 Concentration-response relationship

Cox repeated failures regression on 437 matched PK-VL measurements in 219 ART-naïve individuals (Table 7.3) showed that the hazard of non-suppression decreased by 7% for every 1 mg/L increase in nevirapine C_{min} (95% CI: 2-12%). The estimated probability of non-suppression declined from 26% for a nevirapine C_{min} of 3 mg/L to 18%, 12%, and 9% for C_{min} values of 8, 12, and 16 mg/L, respectively, using the mixed-effects repeated measures logistic model (Figure 7.1a). Likelihood profiling identified a nevirapine C_{min} of 10.2 mg/L (95% CI 7.9–11.8) as most predictive of decreased risk of virological non-suppression (Figure 7.2a in Appendix to Chapter 7). Despite the markedly decreased probability of non-suppression with C_{min} above this threshold and improved specificity and positive predictive value, in comparison to the other C_{min} cut-offs, the identified threshold had inferior sensitivity, accuracy, and negative predictive power (Table 7.4).

7.4.3 Predictors of virological non-suppression

Nevirapine C_{min}, clinical site, age, WAZ, HAZ, adherence, bCD4%, and bVL were all associated with VL>100 copies/mL in univariate analyses ($p<0.2$). However, only C_{min}, clinical site, adherence, bCD4% and bVL were independent predictors ($p<0.05$). After adjusting for these factors, the effect of C_{min} dropped slightly from 7 to 6% (95% CI 1–10%) (Table 7.3). The strongest predictors were adherence and bVL. Every 10% increase is MEMS-score was associated with a 22% reduction (95% CI 10–32%), and a 10-fold higher bVL was associated with a 2.08-fold increase (95% CI 1.50– 2.90), in the risk of non-suppression. Furthermore, for every 10% increase in bCD4%, the risk of viral non-suppression was 29% (95% CI 5–46%) lower. The hazard of non-suppression was significantly greater at 2 of the 3 sites in Uganda, even after adjusting for other significant effects (characteristics by site in Table 7.6 in Appendix to Chapter 7). No significant interactions were detected between predictors in the final model, in particular there was no evidence that associations between NVP exposure and non-suppression varied by centre (Site 1 – ref, Site 2 – p=0.23, Site 3 – 0.51, Site 4 - p=0.09).

Figure 7.1 Change in probability of non-suppression and AEs over the rage of tested neviapine concentrations



Note: Plots present: (a) Probability of non-suppression (VL>100c/mL) for nevirapine C_{min}, (b) probability of transaminase grade 2 or higher elevations for nevirapine C_{min}

Table 7.3 Univariate and multivariate predictors of virological suppression for children in CHAPAS-3 treated with nevirapine

Factor	Univariate*		Final Multivariate Model**	
	HR (95% CI)	p	HR (95% CI)	p
C_{min} [per 1mg/L higher]	0.93 (0.88 – 0.98)	0.004	0.94 (0.90 – 0.99)	0.014
Site [1 ref]	(2) 1.65 (0.91 – 2.99) (3) 1.02 (0.54 – 1.93) (4) 1.41 (0.72 – 2.78)	0.096 0.943 0.315	(2) 1.98 (1.01 – 3.85) (3) 1.19 (0.64 – 2.23) (4) 2.58 (1.15 – 5.75)	0.045 0.573 0.021
Age [per 1 year older]	0.83 (0.71 – 0.98)	0.034		
Pre-ART CD4 % [per 10% higher]	0.82 (0.65 – 1.03)	0.101	0.71 (0.54 – 0.95)	0.019
Pre-ART VL [per 10-fold higher]	2.26 (1.68 – 3.02)	<0.001	2.08 (1.50 – 2.90)	<0.001
WAZ [per unit higher]	0.84 (0.69 – 1.01)	0.069		
HAZ [per unit higher]	0.81 (0.69 – 0.95)	0.013		
MEMS-score [per 10% higher]	0.87 (0.76 – 0.99)	0.037	0.78 (0.68 – 0.90)	<0.001
WHO Clinical Stage [1 ref]	(2) 1.78 (0.81 – 3.91) (3) 1.34 (0.61 – 2.97) (4) 2.36 (0.87 – 6.37)	0.154 0.466 0.090		

Note: *Showing all factors with univariate p<0.2, and hence considered for inclusion in the multivariate model. **Based on backwards elimination using exit p>0.05. HR –hazard ratio, Clinical sites: (1) – University Teaching Hospital, Lusaka, Zambia; (2) – Joint Clinical Research Centre, Kampala, Uganda; (3) – Bristol Myers Squibb Children's Clinical Centre of Excellence, Baylor College of Medicine, Kampala, Uganda; (4) – Joint Clinical Research Centre, Gulu, Uganda. HAZ – height-for-age adjusted Z-scores. WAZ – weight-for-age adjusted Z-scores.

7.4.4 AEs

Skin reactions were rare (four grade-2 HSR, one grade-3 HSR, and one grade-4 SJS). All occurred in ART-naïve patients within 2 weeks of ART initiation, and nevirapine was stopped before PK sampling. The mean pre-ART age and CD4% were 2.8 years and 22%, respectively, and did not differ significantly from other children (ranksum p>0.4); sex was also similar (2 boys, 4 girls; exact p=0.43) as was *CYP2B6*-metaboliser status (3 EM, 2 IM and 1 SM, exact P=0.87).

Transaminase measurements post-baseline were available for 335 children (2273 samples). At enrolment, AST was significantly higher in ART-naïve than ART-experienced children with median 43 IU (5^{th}-95^{th}: 26–127) versus 32 IU (22–60), p<0.001, but ALT was similar with median 21 (9–75) versus 23 (13–53), p=0.14. Transaminase elevations grade 3 and above were rare (15 in total) and were not associated with any particular characteristics (Table 7.7 in Appendix to Chapter 7), there were no cases of acute hepatitis.

Of 39 >grade 1 elevations observed in 235 ART-naïve children, 24 (9 both AST and ALT, 6 AST only and 9 ALT only) were matched with nevirapine concentrations and were included in the Cox repeated failures model. The model identified nevirapine C_{min} (HR per unit higher [95% CI] 1.07 [1.01 – 1.13], p=0.032), but no other factors (including baseline transaminase elevation, sex, age and WAZ/HAZ) to be univariably associated with increased risk of transaminase grade 2 and above elevations. Likelihood profiling identified C_{min} cut-off of 12.4 mg/L (95%CI 7.7-13.5) with HR (95%CI) above vs. below the identified threshold of 5.18 (1.95 – 13.80), p<0.01, (Figure 7.2b in Appendix to Chapter 7). All the observed transaminase elevations were transient and none led to change in treatment, and, although AST and ALT levels were higher in matched samples with nevirapine C_{min} >12.4 mg/L, at most time points the increase from baseline was not statistically significant (Table 7.5). The probability of transaminase elevations by nevirapine C_{min} estimated using mixed-effects repeated measures logistic model are presented in Figure 7.1b, and remained below 10% up to 30 mg/L.

Table 7.4 Comparison of previously published treatment targets applied to nevirapine trough concentrations of the current data set, as well as the thresholds derived in this analysis.

NVP target conc. [mg/L]	2.57 (10th percentile)†		3.0[341]‡		3.5[340]‡		4.04 (25th percentile)†		4.3[342]‡		5.82 (50th percentile)†		10.2†	
HR (95% CI)*	2.05 (1.28-3.30)		1.50 (0.95-2.36)		1.62 (1.08-2.41)		1.62 (1.08-2.41)		1.51 (1.04-2.23)		1.46 (1.02-2.11)		3.05 (1.59-5.86)	
P	0.003		0.078		0.017		0.017		0.032		0.043		<0.001	
Samples with VL>100 c/mL / VL<100 c/mL [n]	< T	> T	< T	> T	< T	> T	< T	> T	< T	> T	< T	> T	< T	> T
	19/17	116/285	20/28	115/274	31/41	104/261	40/55	95/247	45/68	90/234	75/132	60/170	126/241	9/61
Percentage of samples with VL>100 c/mL	52.7%	28.9%	41.6%	29.6%	43.0%	28.5%	42.1%	27.7%	39.8%	27.7%	36.2%	26.1%	34.3%	12.7%
Sensitivity	14.1%		14.8%		23.0%		29.6%		33.3%		55.6%		93.3%	
Specificity	94.4%		90.7%		86.4%		81.8%		77.5%		56.3%		20.2%	
Accuracy	69.6%		67.3%		66.8%		65.7%		63.8%		56.1%		42.8%	
Positive Predictive Value	52.8%		41.7%		43.1%		42.1%		39.8%		36.2%		34.3%	
Negative Predictive Value	71.1%		70.4%		71.5%		72.2%		72.2%		73.9%		87.1%	

Note: < T – below target, > T – above target. *hazard ratio for concentrations below target compared to above target; †current investigation; ‡previously published

7.5 Discussion

We observed that virological non-suppression in a group of African children treated with nevirapine in combination with two NRTIs was affected by nevirapine C_{min} and treatment adherence, as well as pre-ART viral load and CD4 cell count. Despite confirming a significant concentration-response relationship, we could not identify a meaningful exposure cut-off predictive of virological non-suppression. Furthermore, other factors independent of systemic exposures were more strongly associated with non-suppression than nevirapine exposure. Children with lower viral load at ART initiation and better adherence had improved virological outcomes. Adverse events were rare, but high nevirapine C_{min} were associated with transient grade 1 and above transaminase elevations.

Similar to previous investigations in adults[91,340,341] we confirmed that higher nevirapine concentrations led to superior virological suppression in children. Customarily used efficacy thresholds for nevirapine were derived from distributions of concentrations in adult, predominantly Caucasian, patients, even though nevirapine exposures are higher in African populations[358] and children,[305,431] bringing into question their universal applicability.[72] We recently proposed an alternative method of selecting an efficacy threshold based on likelihood profiling and successfully used it for efavirenz in a similar population of African children.[433] Interestingly, a similarly clear cut-off could not be derived for nevirapine, in line with findings by van Leth.[234] The identified C_{min} threshold of 10.2 mg/L, despite having superior sensitivity and negative predictive value, had substantially lower specificity and accuracy than other cut-offs (Table 7.4). In comparison, the threshold identified for efavirenz (C_{min} of 0.65 mg/L) was visibly superior to previously suggested and clearly predicted non-suppression, with only 7% of samples above it but 37% below it having VL >100 copies/mL.[433] Nevirapine has lower potency (protein adjusted IC95 of 196.6 ng/L vs 54.7 ng/L)[64] and shorter half-life than efavirenz, which is the most potent component of NNRTI+2NRTI ART contributing 65% of its total efficacy.[240] A higher contribution to treatment efficacy of the two accompanying NRTIs may have obscured a clear PK efficacy threshold for nevirapine. The above could also explain why virological outcomes were more strongly related to several other factors than nevirapine exposures in children on nevirapine-based ART.

The effects of pre-ART CD4% and VL on virological outcome have been well documented.[61,145,149,150,152,434] In CHAPAS-3, ART-naïve children on nevirapine with a higher pre-ART VL either took much longer to achieve VL <100 copies/mL or never suppressed, consistent with the increased hazard of virological non-suppression with higher pre-ART VL. The pre-ART CD4% in ART-naïve children who rebounded after achieving initial suppression by week 48 was significantly lower than in other groups, and it was also an independent predictor of virological non-suppression. Our

findings highlight the benefits of treatment initiation in early stages of disease, in children with a low VL and high CD4%. The recent START trial[435] in adults confirmed the importance of starting ART early, and supported guidelines recommending initiation of ART regardless of CD4 cell count.[6]

Our findings emphasize the importance of treatment adherence in achieving and maintaining virological suppression, consistent with other studies in African children.[61,152] Children who never achieved VL <100 copies/mL in our study had significantly lower MEMS-scores. Adherence also independently predicted virological non-suppression with risk decreasing by 22% for every 10% higher MEMS-score. It has been hypothesised that adherence above 95% is required to achieve and maintain beneficial effects of ART.[234,436] Interestingly, in CHAPAS-3, the median adherence in children taking efavirenz, an NNRTI administered once a day, was higher than for nevirapine (99% versus 91%).[433] This could explain why the association between adherence and virological outcome was more significant for nevirapine than efavirenz. Meta-analyses confirm that once-daily regimens and reduced pill burden are associated with higher adherence to ART.[437,438] Lower adherence could be a contributory factor to the higher proportion of ART-naïve patients on nevirapine who never achieved VL <100 copies/mL (20% versus 6% on efavirenz) and worse virological outcomes in ART-experienced children. CHAPAS-3 was not designed to compare the effectiveness of nevirapine and efavirenz, but several other studies in children in resource-limited settings suggest better virological outcomes for the latter.[149–154] Yet, nevirapine is currently the only NNRTI formulated as all-in-one paediatric FDC. Whilst developing a similar formulation containing efavirenz could improve treatment adherence and hence virological outcome, this is challenging due to the larger efavirenz dose and higher PK variability due to pharmacogenetics.[415,431]

Adverse events were rare in our study, replicating other paediatric investigations.[107,108,375] High nevirapine concentrations were associated with elevated hepatic enzymes in adults, in particular in those with low BMI,[352,353] but several other studies including African patients showed a low risk of hepatotoxicity.[105,106,358] In CHAPAS-3, while we detected an association between high nevirapine exposures and increased risk of developing >grade 1 transaminase elevations, all observed events were transient and did not lead to ART substitutions. Likelihood profiling identified a C_{min} threshold of 12.4 mg/L as most predictive of these transient events and, although we observed higher transaminase levels during the study when concentrations were above this threshold, they were not significantly different to baseline. Moreover, the baseline values of AST and ALT for ART-experienced children (on nevirapine-based ART for >2 years) were not significantly higher than in ART-naïve children. Together, these suggest that these findings may have limited clinical relevance. Recent reports hypothesise that nevirapine-related hepatotoxicity has a genetic cause.[360,439,440] Similarly HSR

Table 7.5 Transaminase levels at 6, 48 and 96 weeks of treatment, compared to baseline liver enzymes in ART-naïve children, by nevirapine C_{min} threshold most predictive of transient grade 2 and above transaminase elevations

Baseline N = 235		**Nevirapine Conc.**	**Week 6** N = 196		**p†**	**Week 48** N = 200		**p†**	**Week 96** N = 188		**p†**
			Median*	Change from Baseline†		Median*	Change from Baseline†		Median*	Change from Baseline†	
AST [U/L]	43.0 (25.7 – 107.9)	**<12.4 mg/L**	36.0 (24.2 – 95.8)	-5.5 (-8.5 to -3.0)	0.001	37.0 (24.0 – 78.6)	- 5.5 (-8.5 to -2.5)	<0.001	35.0 (25.0 – 71.1)	-7.0 (-10.0 to -4.0)	<0.001
		>12.4 mg/L	42.0 (24.0 – 118)	-7.0 (-38.5 - 27.5)	0.407	51.0 (26.8 – 268.4)	3.8 (-44.0 – 257.0)	0.799	43.0 (31.2 – 205.0)	8.0 (-34.0 – 116.0)	0.488
ALT [U/L]	21.0 (9.0 – 95.8)	**<12.4 mg/L**	22.0 (10.0 – 82.6)	2.5 (0.0 – 4.5)	0.068	26.5 (13.0 – 64.6)	5.0 (2.5 – 8.0)	<0.001	24.0 (8.6 – 67.2)	1.5 (-1.5 – 4.0)	0.373
		>12.4 mg/L	35.0 (9.0 – 66.5)	9.3 (5.5 - 16.5)	0.014	37.5 (18.40 – 215.8)	14.5 (-16.0 – 169.0)	0.441	41.0 (20.2 – 169.0)	16.0 (-11.0 – 186.5)	0.343

Note: *Median (5^{th} and 95^{th} percentile), † Median (95% non-parametric confidence interval) of the differences between week 6, 48 and 96 and baseline, respectively, and p-value from Wilcoxon signed rank test

were rare, possibly due to dose escalation in the first 2 weeks of the study.[107] Small numbers precluded associations with any specific patient characteristics, but they occurred early in the study, before any PK sampling, making it difficult to confirm speculations of their idiosyncratic etiology.[72,347]

Considering nevirapine's low genetic barrier for viral resistance,[342] the risk of non-suppression decreasing with increasing drug concentrations, and the presented safety profile, maintaining C_{min} higher than the current target of 3–8 mg/L could have beneficial effects on general treatment outcomes in African children, and nevirapine concentrations as high as 12.4 mg/L should not lead to increased risk of AEs. Results of recent nevirapine population-pharmacokinetic analysis in children from CHAPAS-3[431] shows that currently recommended paediatric dosage[6] provides average C_{min} at the upper range of the 3-8 mg/L target, even though slow metabolisers determined by *CYP2B6* 516G>T|983T>C genotype are at risk of exposures above 12.4 mg/L.[431]

Our study has several limitations. We could not find a plausible explanation for the detected effect of clinical site on virological outcome, which was not due to small imbalances in other factors since these were either adjusted for or had no association with virological non-suppression. These centre effects likely reflect residual confounding from factors not captured in our study, either differences in other aspects of management on ART or other local variability in the patient populations e.g. in socioeconomic status, distance to clinic etc. However, we found no evidence that the effect of other independent predictors (adherence, VL, NVP exposure) varied across centres (i.e. no interaction/heterogeneity) supporting generalisability of these findings to other settings. No genotyping was conducted at enrolment, so we were not able to assess the impact of pre-existing NNRTI resistance on response. However, we did not find any evidence of an association between pMTCT (predominantly single-dose NVP) and increased risk of non-suppression, similarly to another recent study,[61] suggesting that the impact of pre-existing NNRTI resistance may be relatively small compared to the other factors assessed. Most VLs were matched with nevirapine concentrations measured 12 weeks earlier and one could argue that drug concentrations measured on the same day as VL could be more predictive of virological outcome. However, suppression is likely related to maintained drug exposure above a certain threshold and a random measurement in the time period preceding it could be a better indicator of it. Adherence in our study was only measured in certain time periods and the same drug-taking pattern was assumed to persist until the next measurement. Most children had only 3 VLs after enrolment and our analysis assumed that no viral rebounds occurred in between. Lastly, our findings should not be generalised to ART based on other drugs, in fact, amongst children enrolled to CHAPAS-3, we found different predictors of virological outcome for children on efavirenz.

7.6 Conclusions

Higher nevirapine concentrations were associated with significantly better virological outcomes, but a meaningful cut-off predictive of increased risk of non-suppression could not be identified, possibly due to the effects of the combined NRTIs. Lower VL at ART initiation and higher treatment adherence were the most predictive determinants of virological suppression. The outcome was further affected by pre-ART CD4% and clinical site. Adverse events were rare and, even though we detected an association between nevirapine C_{min} >12.4 mg/L and transaminase elevations, this is of limited clinical relevance due to their transient character. Treatment initiation at lower VL and higher CD4%, increased adherence, and maintaining average C_{min} higher than current target could have a positive effect on virological suppression of African children treated with nevirapine.

7.7 Acknowledgements

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7.8 Author contributions

Designed and conducted the study (V.M., C.K., A.K., D.M.G., D.B., A.S.W., H.M.); assayed the samples (L.W.); provided primary data for use in the analyses (A.C.); designed, conducted and interpreted the analyses (A.B., A.S.W., P.D., H.M.); drafted the manuscript (A.B., A.S.W.); critically revised the manuscript and approved its submission (all authors).

7.9 Conflicts of Interest and Source of Funding

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UK, Ministerio de Sanidad y Consumo, Spain, and the World Health Organization (WHO). Cipla Ltd. donated first-line antiretrovirals. The drug assays were supported in part by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (UM1 AI068634, UM1 AI068636 and UM1 AI106701, U01 AI068632), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and the National Institute of Mental Health (AI068632). The content is solely the responsibility of the authors and does not necessarily represent the official views of any funders.

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7.10 APPENDIX TO CHAPTER 7

7.10.1 Supplementary tables

Table 7.6 Demographic characteristics and model-derived PK parameters of children in CHAPAS-3 treated with nevirapine presented by clinical site

Suppression Group		Site 1	Site 2	Site 3	Site 4	p
Number of patients		49	68	59	44	
Baseline	Age [years]	2.3 (0.8-7.9)	1.9 (0.7-4.4)	1.7 (0.5-2.9)	2.0 (0.8-4.3)	0.020
	Weight [kg]	9.7 (5.9-20.7)	9.0 (6.1-16.3)	9.7 (6.4-13.9)	10.4 (6.4-15.3)	0.224
	CD4% [%]	13.4 (4.5-28.6)	21.0 (9.0-30.5)	18.5 (10.8-32.6)	30.5 (17.0-54.8)	<0.001
	CD4 [cells/ mL]	768 (233-1347)	1075 (433-2977)	1162 (472-3316)	1132 (432-2672)	<0.001
	Viral Load [copies/ mL]	325'980 (24'187-1.1 mil)	440'205 (63'650-8.4 mil)	287'980 (14'347-3.5 mil)	298'887 (3'093-1.3 mil)	0.089
	Sex (M/F)	30/18	34/34	32/27	22/22	0.551
WHO Stage	1	5	16	4	2	0.401
	2	8	24	43	11	
	3	27	24	12	27	
	4	28	4	0	4	
Metabolic Subgroup	EM	15	27	20	12	0.533
	IM	22	27	27	26	
	SM/USM	11/0	14/0	11/1	5/1	
NRTI	d4T	21	24	21	13	0.787
	ZDV	11	20	18	11	
	ABC	16	24	20	20	
PK	C_{min} pm [mg/L]	6.28 (2.10-17.68)	5.68 (2.35-12.96)	5.22 (1.49-11.99)	5.78 (0.86-17.23)	0.714
	AUC pm [mg*L/h]	89.4 (25.7-235.4)	84.2 (35.8-173.9)	77.1 (19.5-166.8)	80.1 (14.1-231.9)	0.584
	C_{max} pm [mg/L]	8.65 (2.41-21.624)	8.19 (3.57-15.91)	7.54 (1.87-15.45)	7.75 (1.34-21.05)	0.454
Adherence	MEMS Score [%]	87.0 (50.0-97.0)	94.0 (81.0-99.9)	86.0 (53.6-97.0)	86.0 (57.3-98.0)	<0.001
	CV C_{min} pm [%]	20.5 (1.0-304.4)	15.0 (2.0-263.4)	15.5 (1.7-627.0)	31.0 (1.3-369.6)	0.547
	CV AUC pm [%]	15.5 (1.9-327.5)	15.0 (2.0-334.7)	18.0 (2.0-760.5)	28.0 (1.0-463.8)	0.499

Note: Presented values are number or median (5^{th} and 95^{th} percentile). Demographic characteristics for 219 individuals ART-naïve at enrolment with matched PK-VL measurements (included in Cox proportional hazards regression model). CV calculated only on individuals who had >1 matched PK visit (182 individuals)

[†]EM (extensive metabolisers)) - 516GG|983TT; IM (intermediate metabolisers) - 516GG|983TC or 516GT|983TT, SM (slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (ultra-slow metabolisers) - 516GG|983CC

Table 7.7 Comparison of characteristic of children in CHAPAS-3 treated with nevirapine with > grade 2 transaminase elevations versus group with no > grade 2 elevations

Transaminase Elevations		> grade 2	No > grade 2	p*
Events / children		15/14	0/321	
ART naïve/ experienced		10/4	225/96	0.99
Sex (M/F)		7/7	169/152	0.98
Baseline	**Age [years]**	2.6 (0.9 – 6.6)	2.6 (0.8 – 8.5)	0.81
	Weight [kg]	10.0 (6.1 – 20.0)	11.6 (6.1 – 22.1)	0.51
	WAZ	-0.9 (-3.6 - 0.3)	-1.6 (-4.3 – 0.1)	0.14
	HAZ	-2.3 (-4.7 - 0.2)	-2.4 (-4.9 - -0.6)	0.69
Metabolic Status	**EM**	4	107	0.83
	IM	6	145	
	SM	4	67	
	USM	0	2	
NRTI	**d4T**	3	103	0.66
	ZDV	6	107	
	ABC	5	111	
PK	**C_{max} [mg/L]**	7.6 (4.1 – 15.7)	8.2 (2.9 – 18.3)	0.83
	C_{min} [mg/L]	4.9 (2.9 – 13.0)	5.8 (2.0 – 15.5)	0.89

Note: Presented values are number or median (5^{th} and 95^{th} percentile). Presented PK parameters relate to daytime exposures. In the > grade 2 transaminase elevations group presented value is the median of nevirapine concentrations measured on the same day or earliest preceding the elevated sample (10 out of 15 matched); in the group with no > grade 2 elevations it is the median of geometric-means of nevirapine concentrations across follow-up calculated within each individual.

*Ranksum Wilcoxon Test or Fisher's Exact Test. M – male, F – female. EM (CYP2B6 extensive metabolisers) - 516GG|983TT; IM (CYPT2B6 intermediate metabolisers) - 516GG|983TC or 516GT|983TT, SM (CYP2B6 slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (CYP2B6 ultra-slow metabolisers) - 516GG|983CC. NRTI – nucleoside reverse transcriptase inhibitor: d4T – stavudine; ZDV – zidovudine; ABC – abacavir.

7.10.2 Supplementary figures

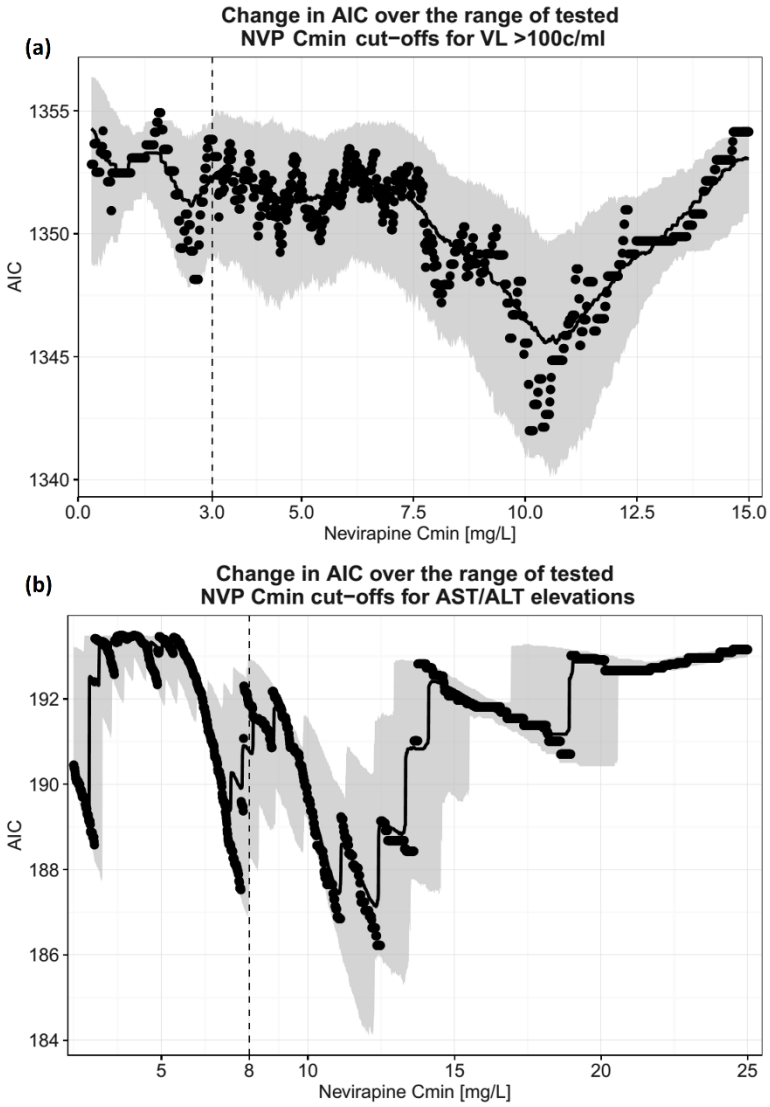

Figure 7.2 Profile likelihoods for virological non-suppression and transaminase elvations for the range of tested nevirapine concentrations

Note: Plots show: a) association between C_{min} and the risk of non-suppression, b) association between C_{min} and the risk of grade 2 and above transient transaminase elevations. The black dots are Akaike Information Criterion (AIC) values for dichotomised cut-offs in tested exposure parameter based on the original data, the black line and shaded grey area are the mean and 95% confidence interval of AIC for cut-offs in the tested exposure parameter from 500 re-simulation runs. Dotted line on Figure 7.2a indicated the current efficacy threshold for nevirapine and on Figure 7.2b - the current safety threshold.

CHAPTER 8: CONCLUSIONS

The introduction of the new paediatric nevirapine and efavirenz tablets, including all-in-one fixed dose combination (FDC) co-formulations of nevirapine with nucleoside reverse transcriptase inhibitors (NRTIs), together with the WHO simplified weight band dosing significantly contributed towards improving antiretroviral (ARV) coverage in HIV-infected children in a resource limited setting by reducing its cost and increasing its feasibility.[33,382,441] With growing clinical evidence new antiretroviral treatments (ARTs) are being introduced in a resource-rich setting, where the cost of healthcare is not a significant constraint. Due to high expense those options are not viable in sub-Saharan Africa, making optimisation of the currently available paediatric treatments a priority. Efavirenz and nevirapine based ARV regimens are the most widely used first line options for children in resource-limited countries. However, studies report high levels of pharmacokinetic (PK) variability for those drugs in children, and sub-optimal exposures (in particular for nevirapine) in the youngest age groups.[33,34,37,40,288,374] In addition, the relationship between systemic exposure to efavirenz and nevirapine and the virological outcome in children has been little studied, and the therapeutic thresholds for nevirapine have never previously been evaluated in African population or children, preventing treatment optimisation. The presented thesis aimed to fill this gap by characterising the PK of efavirenz and nevirapine in African children, with particular focus on the effect of genetic polymorphisms, describing the PK/pharmacodynamics (PD) relationship for those drugs and identifying factors affecting both systemic exposures and overall treatment effect. In addition, by developing a method based on likelihood profiling, we identified drug concentration thresholds most predictive of virological suppression and suggested interventions hypothesised to provide most optimal treatment outcome. The sections below elaborate on the key findings, limitations, recommendations and scope for further work in relation to thesis objectives.

8.1 Synthesis of findings

8.1.1. Is the inter-individual variability in efavirenz and nevirapine concentrations in African children predicted by single nucleotide polymorphisms in *CYP2B6* metabolic pathway alone, or in combination with selected polymorphisms in additional genes (*CYP3A4, CYP3A5, ABCB1, NR1|2, NR1|3*), or other demographic characteristics?

We found that the combined effect of SNPs *CYP2B6* 516G>T and 983T>C on clearance was the main predictor of variability in efavirenz and nevirapine concentrations in African children (Tables 4.3 and

6.3). This confirms previous findings from adult studies (in efavirenz - Chapter 2.3.1.2.1 and nevirapine – Chapter - 2.3.2.2.1) and one recent investigation in efavirenz in South African children.[76] Similar to previous studies[72,176,442] we showed that despite higher prevalence 516G>T variant allele impairs the clearance of both drugs to smaller extent than 983T>C, and even though the latter polymorphism is not present among Caucasian individuals, it is of major importance for black Africans.[29,198] We distinguished 6 separate phenotypic subpopulations in efavirenz pharmacokinetics based on *CYP2B6* 516G>T|983T>C genotype (Table 4.4 and Figure 4.1 right), which could be simplified to 4 different metabolic subgroups as suggested by Dooley *et al*.[71] (Tables 2.3 and 4.4). The combined effect of SNPs 516G>T and 983T>C on nevirapine pharmacokinetics (Table 6.2 and Figure 6.5b) was not surprisingly of smaller magnitude than identified for efavirenz (89% drop in clearance between extensive [EM] and ultraslow metabolisers [USM] for efavirenz versus 68% for nevirapine), what is due to higher contribution of CYP3A4 to total nevirapine biotransformation (Chapter 2.3.2.1). We detected no significant effect of any of the other tested polymorphisms.

The clearance of efavirenz (and hypothetically also nevirapine) has been shown to be affected by a number of other polymorphisms in CYP2B6 pathway but reports in regards to significance of those findings are inconclusive (Chapter 2.3.1.2.1). While SNP 785A>G was previously linked with both 516G>T and 983T>C creating distinguished haplotype groups, its effect was shown to be of small magnitude in comparison with the other outlined polymorphisms. In our study the effect of SNP 785A>G was investigated only in the subset of children from ARROW receiving efavirenz and did not prove significant. The association with SNP 15582C>T identified by Holzinger *et al*.[176] was reproduced in South African patients by Sinxadi *et al*.,[76] nonetheless it similarly was shown to be of limited importance and relevant only for individuals homozygous for common alleles 516G>T and 983T>C. Efavirenz clearance in EM was recently shown to be affected by SNPs *CYP2B6* 18492T>C and 21563C>T,[204–206] which were not explored in our study. Those findings were reported only in Thai patients and to date not reproduced in other populations.

We hypothesised that the PK of the investigated NNRTIs could be further affected by polymorphisms in genes coding nuclear receptors (*NR1*|*2* and *NR1*|*3*) but similar to majority of other studies (Chapter 2.3.1.2.3) did not detect a significant effect of tested SNPs. A recent investigation by Swart *et al*.[84] showed that polymorphisms in CAR (*NR1*|*3*) start playing greater importance only in slow CYP2B6 metabolisers (SM). Slow efavirenz clearance among patients with impaired CYP2B6 metabolism is further affected by polymorphisms in UGT and CYP2A6 accessory pathways (Chapter 2.3.1.2.2) leading to even higher drug exposures. While this effect would be limited only to a small number of patients, it is could explain extremely high efavirenz concentrations documented in a number of case

reports.[177,189,286,443] The polymorphisms in the efavirenz accessory pathways were not explored in our analysis.

In addition to the pharmacogenetic determinants, the PK of efavirenz and nevirapine in our studies (Chapters 4 and 6) was significantly affected by weight, which was accounted for in both models using allometric scaling of clearance parameters and volumes of distribution. The observed average clearance values were relatively higher in younger (and lighter) children than in the older age groups. Similar effect is present for the majority of other compounds and allometric scaling with body weight is currently a widely accepted modelling standard (Chapters 2.3.1.7.3 and 2.3.2.8.3) allowing to account for differences in PK between adults and children resulting from differences in body size.[225,277]

The parameter estimates in both investigations were more accurate under intensive sampling scheme following observed drug intake than under sparse sampling following self-reported intake time. For both drugs this was accounted for as scaling factors on parameter uncertainty. For efavirenz the residual unexplained variability (RUV) in sparse data was twice larger (Table 4.3), for nevirapine we detected an approx. 50% larger uncertainty in RUV and between occasion variability in pre-hepatic bioavailability (BOV F_{preH} - Table 6.3) estimated in sparse data. Even though inclusion of the scaling factors improved the model fit, it is of limited clinical relevance.

In addition our analysis (Chapter 6) showed that nevirapine clearance exhibits a daily rhythm described using a cosine oscillation with peak around 12 noon and amplitude of approximately 29% (Figure 6.3). The relatively large fluctuation in nevirapine clearance has little effect on the estimated daily exposures in terms of C_{min} or AUC (Table 6.2 and Figures 6.7 and 6.8). This could be speculated due to long terminal half-life of nevirapine. Since the daily nevirapine dose is split between morning and evening administration the variability in C_{min} resulting from the diurnal variability in clearance is much smaller than differences in exposures resulting from *CYP2B6* polymorphisms. The small magnitude of this effect on nevirapine concentrations could explain why the diurnal oscillation in nevirapine clearance was never previously characterised.

No age effect (maturation) was detected on clearance in either of the drugs but we identified age-driven differences in nevirapine F_{preH} (at birth estimated as 58.3% of the value in older children [reference fixed to 100%], 90% of F_{preH} was reached by age of approximately 3.3 years and the half-life of the process was 1.55 years [Figure 6.4]). Similar age-driven differences in nevirapine bioavailability were previously described by Foissac *et al*.[374] Mechanistic hypotheses contributing to this effect are discussed in Chapter 6.5. Our finding does not exclude the presence of maturation in nevirapine clearance in younger children, nonetheless in our data this effect was overshadowed by

the effect on F_{preH}. On the contrary, the recent analysis by Salem *et al*.[162] confirms that in children older than 3 years no further maturation in efavirenz clearance should be observed.

The shringake in the presented population PK models for estimated variability parameters is outlined in Table 8.1. For efavirenz the values of shrinkage for BSV in CL and F1 are acceptable but not suprising they are much higher for the BOV parameters. The number of sampling occasions for children included in the analysis ranged between 2 and 8 and contributed to high shrinkage values for occasions with limited number of measurments. Similar trend in shrinkage can be seen for nevirapine, although the estimated shrinkage in BSV for pre-hepatic bioavailability was relatively high (37%). This could be speculated due to high complexity of the model and high number of fixed effects relating to this parameter. The presented shrinkage estimates indicate that the model paramters Empirical Bayes Estimate were estimated with acceptable precission.

	Virability parameter	Efavirenz	Nevirapine
BSV	Clearance	19.1 %	26.2 %
	Bioavailibility*	24.2 %	37.0 %
BOV	Bioavailibility*	4.7 – 28.9 %	7.7 – 44.7 %
	Mean transit time	39.7 – 67.6%	33.0 – 66.1 %
	Absorption rate constant	55.5 – 86.3 %	53.2 – 84.9 %
	Clearance	52.0 – 66.4 %	------
RUV		31.5 %	36.1 %

*Table 8.1 Overviewe of shrinkage in variability parameters in the population pharmacinetic models presented in Chapters 4 and 6. *For nevirapine this relates to pre-hepatic bioavailability.*

8.1.2. Do average efavirenz and nevirapine exposures differ between WHO 2010 dosing weight bands and different metabolic subgroups, determined by individual CYP2B6 genotype, and could genotype based dose adjustment provide more balanced exposures between metabolic subgroups in African children?

WHO 2010 weight band dosing provided adequate average efavirenz exposures across all weights (Figure 4.1a). Nonetheless, noticeable differences were observed between different *CYP2B6* genotype subgroups irrespective of their weight (Figure 4.1b). CYP2B6 EM had average efavirenz exposures at the bottom of the current therapeutic range of 1 – 4 mg/L putting them at an increased risk of virological failure, whereas SM and USM had mid-dose concentrations above the target range putting

them at an increased risk of developing CNS AEs. Based on results of pharmacokinetic simulations we suggest that a dose adjustment strategy based on individual *CYP2B6* 516G>T|983T>C genotype (Table 4.5), should ensure more balanced efavirenz exposures between different metabolic subgroups in all weight bands (Figure 4.2). The suggested dosage algorithm maximises the use of the new efavirenz double-scored paediatric 600 mg tablets.

Similar genotype based dose adjustment approaches were previously suggested in adults (Chapter 2.3.1.6) and children (Chapter 2.3.1.7.7) but were guided solely on the individual 516G>T genotype. We showed that such approach could lead to supratherapeutic efavirenz exposures in individuals with 983T>C variant allele (Figure 4.3) and that despite a lower prevalence this SNP should be taken into consideration to guide dose adjustments in African children. This was also postulated in a recent study in South African children.[286] Implications of the suggested dose optimisation approach are further discussed in Chapters 8.3 and 8.4.

The average nevirapine exposures obtained under WHO 2010 weight band dosing was adequate in majority of children (Figure 6.5a) with exception of infants weighing 4 – 6 kg (where >25% of children had evening C_{min} < 3mg/L). This effect was driven mostly by EM and intermediate metabolisers (IM) (43% and 26% <3 mg/L,[95] respectively - Figure 6.5b). In addition, the average nevirapine C_{min} differed significantly between metaboliser groups in all tested weight bands with majority of SM and USM having exposures above the current upper therapeutic target of 8 mg/L.[95] To prevent suboptimal nevirapine concentrations we suggest the daily dose for EM and IM in the lowest weight band to be increased from 100 to 150 mg. Further harmonisation of nevirapine exposures could be achieved by 50% reduction of nevirapine dose in SM and USM in all other weight-bands. Implications and feasibility of genotype guided treatment optimisation for nevirapine are discussed in Chapter 8.3.

8.1.3. Are efavirenz and nevirapine concentrations predictive of virological response and adverse events in African children treaded with paediatric solid FDC formulations, and what is the contribution of other variables to the observed treatment effects?

The results of the conducted PK/PD analysis (Chapter 5) suggest that efavirenz exposures are the main predictor of the virological outcome in African children on efavirenz based ART. There was a strong association (P<0.0001) between the systemic exposure to efavirenz (measured as C12h, C24h or AUC)

and the risk of non-suppression (lower risk of VL > 100 copies/mL for higher exposures), which was best described using a log-linear model (Table 5.2). After adjusting for the effect of all significant covariates in the multivariate analysis we identified that sex, age and site as additional independent predictors of virological outcome (with an interaction in the effect of the first two variables, Table 5.3). The risk of virological non-suppression for boys <8 years was 5 times greater than that for girls of similar age. Older children had increased risk of virological non-suppression compared with younger children, but there was no evidence of a difference between boys and girls >8 years. The hazard of virological non-suppression was significantly higher in the smallest site, which contributed only 5 children and is of little clinical relevance. There was marginal evidence that poorer adherence independently increased the hazard of virological non-suppression (P = 0.065 after including in the final multivariate model in addition to significant differences between suppression groups presented in Table 5.1). The AEs in children in efavirenz arm were rare and could not be associated with any particular characteristics.

The association between plasma nevirapine exposure and the virological outcome (explored in Chapter 7) was considerably weaker than for efavirenz (also inversely correlated), and in the multivariate analysis pre-ART VL (2.08-fold increase in risk for 10-fold higher bVL, P<0.001) and adherence (22% reduction in risk for every 10% increase is MEMS score, P<0.001) were the strongest independent predictors of non-suppression (Table 7.3). The risk of non-suppression was in addition higher for children with lower baseline CD4% (every 10% increase in bCD4% was associated with a 29% drop in the hazard). Similar to efavirenz there was a significant effect of treatment site, but after accounting for all independent predictors there was no association with age or sex. The hypersensitivity reactions to nevirapine were rare and could not be attributed to any characteristics, but transient transaminase grade 2 and above elevations were associated with high nevirapine concentrations (7% increase in risk for every unit increase in nevirapine C_{min}, P=0.032).

The associations between drug exposures and treatment outcome for efavirenz in children have been little studied and for nevirapine never been previously characterised. Most previous paediatric investigations focused on predictors of treatment efficacy other than drug exposures (Chapter 2.3.1.7.5 and 2.3.2.8.5) and the majority of them were comparative studies including 2 or more different treatments (Table 2.6 and 2.7). Interestingly our analyses, in addition to systemic exposures, found different predictors of virological outcome for both of the investigated drugs (Tables 5.3 and 7.3). Nonetheless, it could be speculated that sex and age effects detected for efavirenz could be linked to differences in treatment adherence between age groups and genders (and hence the effect of adherence itself for efavirenz proved not significant). Literature shows inconclusive information on the effect of gender and age on treatment outcome with some studies reporting increased risk of

treatment failure for younger children,[280,444,445] some for older age groups[149,153] and some a non-linear effect.[154] Conversely, the reports consistently show that treatment failure is linked to malcompliance,[61,149,152] and it has been speculated that adherence above 95% is required to achieve and maintain virological suppression.[234,436] Considering that a large proportion of children in the efavirenz arm took > 95% of the prescribed doses, it could provide a plausible explanation why the effect of adherence for that drug was not picked up in our analysis. Differences in treatment compliance could also explain better virological outcome observed among children on efavirenz than nevirapine in CHAPAS-3 (the average MEMS scores higher [Tables 5.1] and lower [Tables 7.1 and 7.2], respectively).

Even though CHAPAS-3 was not designed to compare the effectiveness of nevirapine versus efavirenz, a number of other studies suggest a better virological outcome for the former (Table 2.6). Meta-analyses report that once-daily regimens and reduced pill burden are associated with higher adherence to ART.[437,438] Interestingly, in CHAPAS-3 the median adherence in children taking efavirenz, an NNRTI administered once a day, was higher than for nevirapine (99% versus 91%). Lower adherence could be a contributory factor to the higher proportion of ART-naïve patients on nevirapine who never achieved VL <100 copies/mL (20% versus 6% on efavirenz), as well as worse virological outcomes in ART-experienced children. Taking presented arguments into consideration one could speculate that once-daily regimens could be associated with superior virological suppression than twice-daily (although it cannot be excluded that differences in suppression levels seen in our study were related to differences in baseline CD4%, VL and age between the treatment arms).

We found that children treated with nevirapine with higher baseline CD4% and lower baseline VL had a better virological outcome but surprisingly a similar effect was not detected for efavirenz. We hypothesise that this could be resulting from differences in baseline characteristics and smaller sample size in the efavirenz arm. According to WHO 2010 guidelines[11] (followed in CHAPAS-3) nevirapine is licensed for use from 3 months of age, while efavirenz from 3 years, which contributed to a higher average age in the efavirenz arm. In CHAPAS-3 children in the efavirenz arm were older with lower baseline VL and marginally higher CD4%. Those factors together with a smaller number of children in efavirenz group could have precluded us from detecting similar predictors of virological suppression as for nevirapine. Nonetheless, better treatment outcomes in children initiated on NNRTIs at higher baseline CD4% and lower baseline VL have been previously highlighted in a number of investigations (Tables 2.6 and 2.7) as well as additional trials including other treatment options.[12,444–446] Recently, studies START[435] and TEMPRANO[447] reported benefits of early ART initialisation in HIV infected asymptomatic adults in line with our findings in children, which are consistent with results of paediatric study CHER.[446]

Lastly, one could deliberate on the selection of trough concentrations as the PK predictor of treatment effect in presented analyses. Our main criterion was applicability of research to the clinical settting. Even though efficacy and/or safety could be correlated which various other PK parmaters such as: CL, AUC, and C_{max}, only trough (or mid-dose) drug concentrations are routinely measured in clinical practice (TDM), wherea the other paramters need to be derrived limiting their application. It has been speculated that AUC is a better descriptive measure because it captures drug exposure within a certain time frame (dosing interval), nonetheless taking into consideration the mechanism of action of ARVs virological non-suppression is caused by drug concentrations below a defined threshold (required for maintaining inhibition of viral replication) and C_{min} might be better indicator for it. Pfister *et al.*[172] correlated apparent clearance (CL/F) with probability of virological failure, and even though this measure is more sensitive to temporal changes in adherence patterns than clearance, physiologically it is not expected to change much between sampling occasions. C_{min} is a measure more sensitive to suboptimal treatment adherence. In contrast AEs are customarily associated with C_{max}, and even though from mechanistical point of view this parameter might be more appropriate, nonetheless it is not observed routinely in clinical practice. Additionally, the parameters used in our analyses were model derived and we observed very high correlation between them (Chapter 5.4). All analyses were repeted using different PK predictors and interpretation of the results was the same (not shown).

8.1.4. What thresholds in efavirenz and nevirapine concentrations are most predictive of virological suppression and adverse events in African children?

The developed method for selection of drug exposure cut-off most predictive of increased risk of clinical effect (here virological non-suppression or AE) based on likelihood profiling and Cox proportional hazards model identified mid-dose concentration (C12h) of 1.12 mg/L, trough concentration (C24h) of 0.65 mg/L and AUC of 28 mg•h/L as efficacy thresholds for efavirenz in African children (Table 5.2 and Figure 5.1). While the new C12h cut-off did not differ markedly in sensitivity, specificity, or negative predictive power from the 1.0 mg/L value proposed by Marzolini *et al.*,[94] the proposed cut-offs for C24h and AUC substantially improved specificity, accuracy and positive predictive power, while maintaining a negative predictive power comparable with previously suggested therapeutic thresholds (Table 5.4).

The low frequency of CNS AEs in our study prevented identification of the upper therapeutic threshold for efavirenz. We suggest the efavirenz C12h efficacy threshold in children should remain 1 mg/L, but based on the results of our investigation we suggest that the targets for C24h and AUC could be lowered to 0.65 mg/L and 28 mg•h/L, respectively. Due to the insufficient evidence in our study we suggest that the upper therapeutic threshold should remain 4 mg/L as previously suggested.[94,95]

Even though the risk of virological non-suppression decreased with increasing nevirapine C_{min}, there was no clear C_{min} threshold predictive of virological outcome. Likelihood profiling identified a nevirapine C_{min} of 10.2 mg/L as most predictive of decreased risk of virological non-suppression (Figure 7.2a) and while it had superior sensitivity and negative predictive value, the specificity and accuracy of this cut-off was substantially lower than for previously suggested alternatives (Table 7.3). In fact, none of the nevirapine C_{min} thresholds presented in Table 7.3 provided a meaningfully better predictive power. This could be explained by the lower potency of nevirapine than efavirenz, and as a consequence lower relative contribution to the total antiretroviral effect, which is to greater extend affected by the concentrations of companion drugs.

Likelihood profiling applied to the Cox model describing the association between nevirapine concentrations and the risk of developing grade 2 and above transaminase elevations identified C_{min} of 12.4 mg/L as threshold most predictive of those events (Figure 7.2b). Nonetheless, the probability of transaminase elevations estimated using mixed-effects repeated measures logistic model showed that the risk remained below 10% for nevirapine concentrations up to 30 mg/L (Figure 7.1b). Additionally, the vast majority of the transaminase elevations in our study were transient and resolved without any intervention (including all but one of the 15 grade 3 and above elevations). The observed transaminase elevations for individuals with nevirapine concentrations > 12.4 mg/L were small (Table 7.5) and of limited clinical importance. Based on those findings we suggest that maintaining average nevirapine C_{min} at the upper range or above of the current target could have a positive effect on virological suppression in African children without increasing toxicity.

PK/PD evaluation in further clinical cohorts including different populations should be done to validate our findings.

8.2 Significant contributions to the field of HIV research

In the first study (Chapter 4) we confirmed that SNPs *CYP2B6* 516G>T and 983T>C are the main predictors of variability in efavirenz concentrations and were the first to quantify their effect on clearance in children and average efavirenz exposures in dosing weight bands suggested by WHO. We showed that dose adjustment based solely on SNP 516G>T could lead to significant overexposure in approximately 14% of African patients with 983TC or 983CC genotypes proving that dosing guidelines in African children should take into consideration the effect of both of those polymorphisms.

In our second investigation (Chapter 5) we described the log-linear relationship between efavirenz concentrations and risk of non-suppression and hypothesised that some of the previous studies were unable to identify this effect because they were underpowered, limited to a single measurement per patient, or utilised inadequate statistical methods. In addition, we suggested a new approach based on likelihood profiling and Cox repeated failures model to define concentrations thresholds associated with increased risk of virological non-suppression for efavirenz in African children. We derived new efficacy cut-offs for efavirenz trough concentrations and AUC (which in our study proved superior in predicting treatment outcome than previously suggested thresholds) and propose they should be utilised for optimisation of HIV treatment in African children.

The third study (Chapter 6) is the first report of the effect of 983CC homozygosity on nevirapine pharmacokinetics, the first paediatric investigation describing the effect of SNP 983T>C on nevirapine concentrations, and the first study in nevirapine describing combined effect of SNPs 516G>T and 983T>C using metabolic subgroups. Our analysis is also the first to date to characterize the diurnal oscillation in nevirapine clearance and to evaluate the effect of this phenomenon on systemic drug exposures through simulations. Additional novelty is implementation of the hepatic first pass model allowing to distinguish between pre-hepatic and hepatic sources of variability in bioavailability, which was never previously used in this drug. Even though our findings have limited clinical relevance they increase the knowledge of nevirapine pharmacokinetics.

Our fourth study (Chapter 7) is the first study characterising the relationship between nevirapine concentrations and virological suppression and transaminase elevations in children. In addition our analysis suggests beneficial effect of ART initiation at lower VL and higher CD4%, and higher ART adherence on the virological suppression.

8.3 Implications for the current HIV treatment guidelines

We suggest that *CYP2B6* genotype guided efavirenz dose adjustment should provide a more optimal treatment outcome in African children. We base this recommendation on the strong link between efavirenz exposures and the virological outcome detected in our study (Chapter 5) and in previous investigations (Chapters 2.3.1.4, 2.3.1.5, 2.3.1.7.5 and 2.3.1.7.6). In addition, our analysis (Chapter 4) shows that the combined effect of SNPs 516G>T and 983T>C is the main predictor of variability in efavirenz PK, which confirms the previous studies (Chapters 2.3.1.2, 2.3.1.7.3, 2.3.1.7.4). Our findings (Chapter 4) indicate that under current dosing schedule[6] African children EM for CYP2B6 are under increased risk of developing suboptimal drug exposures, which could hypothetically lead to treatment failure. In contrast, children SM and USM for CYP2B6 exhibit high efavirenz exposures, which can contribute towards CNS adverse events and have been associated with treatment discontinuation in adults (Chapter 2.3.1.5) and case reports of severe behavioural changes and CNS manifestations in children.[286]

Our recommendations contradict conclusions drawn from the population pharmacokinetic modelling and simulations conducted by Bristol-Myers Squib[291] supporting Sustiva (efavirenz originator drug) label extension to children < 3 years old granted by FDA in 2013[268] and EMA in 2015.[269] The analysis by Luo *et al*.[291] presented some conflicting findings – despite detecting a significant reduction in efavirenz clearance determined by the individual *CYP2B6* 516G>T genotype authors concluded that *CYP2B6* genetic status was not informative in guiding paediatric dosing due to large variability in clearance within the genotypic subgroups leading to disregard of *CYP2B6* effect in approved Sustiva dosing recommendations. We would like speculate that those findings were confounded by the inherent flaws in the analysis and the model and would like to discuss them further.

The aforementioned investigation inadequately characterised sources of variability in efavirenz PK. The authors stressed the importance of developmental changes in youngest children, nonetheless the age effect was not accounted for in their model. Additionally, the differences in clearance values between metaboliser subgroups were presented with disregard to children's age (each subgroup included children ranging between 3 months to 18 years old). Recent analysis by Salem *et al*.[162] (Chapter 2.3.1.7.3) reported significant differences in efavirenz PK driven by age (maturation of efavirenz clearance and age-driven differences in bioavailability of liquid formulation). To avoid the confounding effect of age Luo *et al.* should have compared the differences in clearance between metaboliser subpopulations by weight (or age) bands, or alternatively based the comparison on systemic exposures to efavirenz. Furthermore, in our analysis we showed that SNP *CYP2B6* 516G>T alone was not sufficient to determine metaboliser phenotype for black African children, and that SNP

983T>C despite lower prevalence affects efavirenz clearance to greater extend. The latter SNP was not taken into account in the analysis by Luo *et al*., even though over 50% of children in that study were black African.

Additionally, the clearance estimated in the discussed study was expressed as apparent clearance (CL/F), which by definition is affected by the bioavailability. If the variability in the bioavailability is not accounted for in the model, it will inflate the variability in CL/F. It is unclear, if authors explored any covariates other than formulation on bioavailability, and did not include any between subject variability (BSV) or between occasion variability (BOV) on this parameter (despite analysing longitudinal data with repeated sampling). Karlsson *et al*.[390] showed that ignoring BOV might lead to biased parameter estimates and high incidence of spurious findings. It can be speculated that the variability in the data was further inflated by lack of accurate dosage history. The overall poor performance of Luo's model is apparent by extremely high values of residual unexplained error (proportional error of 45% for capsules and 78% for solution), as opposed to 6.7% proportional and 0.1 mg/L additive residual error in our model.

We argue that the aforementioned shortcomings of the outlined analysis contributed to confounded conclusion and the current treatment guidelines in African children should be re-evaluated and optimised between the metabolic groups determined by individual *CYP2B6* 516G>T|983T>C genotype. Our recommendations are in line with the recent guidelines for efavirenz dosage in children < 3 years developed by the Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children at the United States Department of Health and Human Services (DHHS) (Chapter 2.3.1.7.7 Table 2.8) currently tested in study P1070[289] (discussed in more detail in Chapter 8.5).

Despite a similar strong link between pharmacogenetics and variability in nevirapine exposures (Chapters 2.3.2.2.1 and 6), our findings indicate that interventions other than dosage modification could ensure a more beneficial virological outcome. Our analysis showed that CYP2B6 EM and IM in the lowest weight band had exposures below the current therapeutic range, whereas SM and USM in all other weight-bands had concentrations above it. Even though the literature highlights that suboptimal nevirapine concentrations might lead to emergence of drug resistance and increased risk of virological failure,[30,33] in our analysis no clear efficacy cut-off could be defined (Chapter 7). While dose increases in CYP2B6 EM and IM in the lowest weight band could hypothetically improve virological outcome, implementation of this strategy would require addition of nevirapine liquid to the current formulation, increasing the cost of treatment and limiting its feasibility. Additionally, given the fast growth rates in infants after starting ART they would spend only a short time within that weight band what would limit the effect of suboptimal exposures.[37] Conversely, the dose reductions

in CYP2B6 SM and USM would not be possible while treating with all-in-one paediatric FDCs and would impose children to receive two separate tablets. This would not only increase the cost of ART but also hypothetically could affect adherence by increasing the pill burden. Furthermore, the results of our study showed a benefit of maintaining nevirapine exposures in children at the top or above the current upper therapeutic limit of 8 mg/L[95] without increasing toxicity (Chapter 7).

The detected diurnal variability in nevirapine clearance had little effect on the average drug exposures and does not require modifications of the current dosing schedule, but the results of the PK simulations indicate that when the daily nevirapine dose cannot be split equally, larger doses should be given in the morning (Figure 6.7 and Figure 6.8).

In addition, even though we detected a concentration-response relationship for nevirapine this correlation was not as strong as for efavirenz (Chapter 7). In fact, our analysis showed that other factors (in particular treatment adherence and baseline VL) were stronger correlated with virological outcome than nevirapine concentrations (Chapter 7). Based on our findings we hypothesise that interventions improving treatment compliance and ART initialisation at a lower baseline VL and CD4% could provide a more beneficial effect on warranting the most optimal outcome of treatment with nevirapine than modification of the current dosage guidelines. Such interventions should similarly provide further improvements of treatment outcome for efavirenz in addition to genotype guided dose adjustment.

8.4 Implications for HIV research and clinical practice

Few studies evaluated genotype based dosage adjustment strategies in clinical practice. Efavirenz dose reductions guided by individual genotype or phenotype proved to limit drug toxicities without compromising treatment efficacy in adults (Chapter 2.3.1.6) but to date only scarce information is available in children (Chapter 2.3.1.7.7). Paediatric data is currently limited to preliminary results of study P1070.[289] We showed that efavirenz dose optimisation based solely on SNP 516G>T, investigated previously in adults and currently in children in study P1070,[290] would not be appropriate in African children, as it would lead to overdosing in individuals with 983T>C variant allele. No prior study evaluated the effects of efavirenz dose adjustment based on the allocation to metabolic CYP2B6 subgroups guided by combined 516G>T|983T>C genotype and we identify this to be the area of the greatest clinical need. One could envisage that implementation of such strategy in a recourse-limited setting would be hindered by access to genotyping and its cost. In order to facilitate such "apriori" dose adjustments a "point of care" genotype testing system would need to be developed. Implications of efavirenz "apriori" genotype-guided dosage (based on combined effect of SNPs 516G>T and 983T>C) for treatment efficacy, safety, feasibility and cost should assessed though a well-powered clinical study in African patients. Hopefully some of those questions will be answered by the results of the ongoing study P1070.[290]

The results of our study additionally put in question the universal efavirenz dose reduction strategy (from 600mg to 400mg) in adults postulated by ENCORE-1 study team and recently incorporated into WHO guidelines.[6] The universal dose reduction was previously challenged by Maartens and Meintjes,[266] who highlighted possible alterations to efavirenz pharmacokinetics in pregnancy, in patients on rifampicin-based treatment for tuberculosis, and in different metabolic subgroups. Recent study by Dooley *et al*.[71] confirmed that pregnancy indeed increases the efavirenz clearance by on average 19%. Our study, similar to Lam *et al*.[267] and Hui *et al*.[223] argues that dose reduction based on genotype may be a more practical approach for optimizing therapy. The simulation study by Lam *et al*.[267] reported that individuals 516GG would be at an increased risk of suboptimal concentrations on the new dose of 400 mg comparing to standard 600 mg and suggested that 500mg would be more optimal for those patients. The main predicament of ENCORE-1 study was that efavirenz dose reduction from 600mg to 400mg would reduce the cost and improve safety of treatment without compromising the efficacy. Lungren *et al*.[131] hypothesised that a universal dose reduction to 400mg could provide a cost saving of $14 per year for patients on FDC tablets, but a cost-effectiveness study by Schackman *et al*.[263] suggests that "apriori" *CYP2B6* genotyping to guide efavirenz dosing would also provide similar cost savings.

A lot of controversy surrounds the current efavirenz efficacy threshold of 1 mg/L[94,95] and a number of studies hypothesise that the actual cut-off should be lower. In addition the 1 mg/L cut-off was derived based on random efavirenz mid-dose concentrations from therapeutic drug monitoring (TDM), nonetheless they are customarily applied also to trough concentrations. Our study derived new, alternative trough concentration and AUC efficacy thresholds for African children but there is a need for similar investigations in adults. Recently we applied our method to adult data from a small South African study and showed that a TDM efavirenz concentration of 0.7 mg/L was most predictive of increased risk of non-suppression.[236] The results should be interpreted with caution due to a small sample size and our findings should be confirmed in a larger, longitudinal trial with repeated measurements. The derived paediatric and adult target thresholds should be verified in a prospective clinical study.

The CNS AEs in our study for efavirenz were very rare, similar to other paediatric investigations, and we hypothesise that is due to underreporting and lack of appropriate tools facilitating their detection. Systemic reviews suggest that the scale of neurological and neuropsychiatric impairment caused by efavirenz treatment might be underestimated.[245,251] In addition patients develop tolerance to experienced CNS AEs soon after start of efavirenz treatment.[69,186] A study by Ciccarelli *et al*.[250] reported that almost 50% of otherwise asymptomatic patients on efavirenz had some form of cognitive disorders or impairment. Such neurocognitive complications of treatment have been little studied in children and should be of great concern, as they could affect not only their well-being but also their neuropsychological and behavioural development as well as future educational achievements. It could be speculated is that in the majority of children, due to development of tolerance to the common CNS AEs, such behavioural and cognitive changes might remain unnoticed. Alarming are also the recent reports of severe CNS manifestations, such as cerebellar dysfunction, seizures, aggressive and anti-social behaviour in South African children treated with efavirenz, which were all associated with impaired CYP2B6 metabolism (caused by 516G>T and 983T>C variant alleles).[286] Those reports strengthen the importance of interventions aiming to reduce prevalence of persistently high efavirenz exposures in African children and their authors, similar to us, postulated that such dose adjustments should be guided by the individual 516G>T|983T>C genotype. Nonetheless, more research is required to establish the extent of cognitive impairment in children treated with efavirenz and underreporting related to lack of appropriate measurement tools.

Recently, following a WHO initiative, a discussion started on development of efavirenz based all-in-one paediatric FDC tablet.[448] It is expected that similar to currently available paediatric nevirapine FDC tablets such formulation would provide not only cost savings, improve the feasibility and coverage of antiretroviral treatment in children in resource limited setting, but also reduce pill burden related ART.

Efavirenz PK data from ARROW and CHAPAS-3 (analysed in this thesis), with addition of PK data from other paediatric studies, were used to create a paediatric efavirenz mega-model and simulate exposures of various dosage scenarios across WHO weight bands.[448] While we acknowledge the advantages of such formulation, taking into consideration the results of our analysis we question its universal roll without prior genotyping. Furthermore, we emphasize that the currently available paediatric formulations (ABC/3TC FDC and double scored efavirenz tablet) give more flexibility in adjustment of efavirenz dose based on individual *CYP2B6* genotype preventing exposure to excessive concentrations. In addition, if efavirenz is administered with abacavir containing NRTI-backbone this already reduces the frequency of drug intake from twice a day (BD), required for nevirapine based regimen or other NRTI-backbones, to once a day (QD). Substitution of a BD with a QD regimen has been shown to have a positive effect on treatment compliance,[437,438] which is also observed in CHAPAS-3 as higher adherence scores for efavirenz (despite a 2-pill regimen). We hypothesise that an efavirenz-based QD 1-pill regimen would have little advantage over a QD 2-pill regimen, but the latter could allow dosage personalisation. Until more conclusive data on the effect of sustained high efavirenz concentrations on behavioural and cognitive impairment in children is generated, our priority should be safeguarding this vulnerable population from possible but omittable risks. The modification of current guidelines cannot be however implemented based on the results of our analysis alone and should be supported by the results of a prospective study.

The results of such randomised prospective study led to the recent changes in the recommended first line regimen for children <3 years of age.[6,111] Study P1060[145,146] showed significantly lower mortality and higher suppression in children <3 years old on a lopinavir boosted with ritonavir (LPV/r) rather than nevirapine based ART. Nonetheless, the use of nevirapine in this age group still has some advantages comparing to other available treatment options. It is the main drug used for pMTCT worldwide and it can be hypothesised that a continuous treatment with nevirapine following the single dose at birth could help prevent the establishing of HIV latent reservoirs in neonates[449] and possibly recreate phenomenon observed in the case of Mississippi baby.[450] With a rapid progress of work on HIV cure HIV-infected children with virtually undetectable viral load and limited viral reservoirs would stand the best chance to have the virus eradicated from their bodies. Even though more studies are required this is currently the fastest evolving branch of HIV research.[451] Much controversy surrounds early treatment start in children due to lifelong exposure and concerns about the toxicity but recent results of study CHER[446] proved the benefit of such ART initiation approach. Furthermore, if the treatment start at birth could facilitate eradication of the virus in the future life, when this happens those children would require no further antiretroviral treatment. Currently this is a hypothetical scenario but due to low cost and availability of age appropriate formulations treatment

with nevirapine from birth could help facilitate it in the future. Additionally, in terms of older children a number of studies showed that nevirapine re-use in patients who achieved and maintained virological suppression on LPV/r based regimen is a safe option maintaining the virological outcome and hypothetically preventing the long term toxicities related to LPV/r. [58,59] The pharmacokinetic simulations we conducted showed that (with minor modifications) the current simplified weight-band dosing provides optimal nevirapine exposures in children weighing >4kg justifying further use of the current paediatric FDC formulation, which showed high acceptability among children and the caregivers.[158]

8.3 Limitations of the studies

The main limitation of the efavirenz pharmacokinetic study (Chapter 4) is that it was restricted only to a selection of SNPs which didn't include polymorphisms in the accessory metabolic pathways. Recent reports show that polymorphisms in the accessory pathways become particularly important when primary metabolic route is affected[103,177,452] and could help predict individuals with exceptionally high efavirenz concentrations. In addition literature shows that absorption of efavirenz is increased by food co-administration, which was not recorded in our study, which potentially increased the levels of between occasion variability in the absorption parameters and bioavailability. Small number of TB co-infected children in our study hindered drawing significant conclusions to the effect of TB treatment on efavirenz pharmacokinetics.

The majority of the data included in the nevirapine pharmacokinetic analysis (Chapter 6) was sparse with a self-reported intake time, which might not be accurate. This could have inflated the variability in the absorption parameters and the detected diurnal effect what we tried to minimise by exclusion of outlying samples, ones with uncertain dosage history and below level of quantification (BLQ). It could addtionaly be speculated that the unequal interval between morning and evening drug intake could have inflated the characterised diurnal variation. The applied non-linear mixed effects modelling should account for differences in the dosing intervals but only, if those were recorded correctly. Furthermore, there could be alternative explanations to the detected diurnal effect. In some drugs it was contributed to differences in hepatic blood flow,[322,323] which could be affected by food intake (not recorded in our study). Nonetheless, during the model building diurnal effect provided better fit when included on clearance rather than bioavailability (based on the changes in OFV and diagnostic plots). An additional confounding factor might be that both analysed studies had a different procedure for splitting of unequal daily doses (higher dose in the evening in CHAPAS-1 and in the morning in CHAPAS-3), but the model-based approach we employed should have account for this difference. Lastly, we

did not have genotype information for children from CHAPAS-1 study and used mixture modelling to allocate those individuals to different metabolic subgroups.

The limitation of the method developed for estimation of a dichotomized efficacy threshold in the second and fourth studies (Chapters 5 and 7) is that it might lead to over fitting and low external generalizability (which we were unable to test in a validation data set). In addition the CIs for the identified thresholds were relatively large which could be contributed to a small data set. Larger studies are required to facilitate a more precise estimation of the efficacy cut-off and a prospective study is needed to confirm it. While our findings should also be interpreted in terms of treatment effectiveness and a true efficacy threshold in a setting of ideal adherence might be lower, treatment effectiveness measure is more relevant to a clinical setting. Adherence in our studies was measured only in certain time periods and MEMS scores were extrapolated to periods when patients did not have devices assuming no changes in drug taking patters which could have affected our findings for this variable. VLs in our studies were not measured between baseline and week 36 what prevented us from characterising the initial VL decline after treatment initialisation. Furthermore, VL was measured on average only up to every 24 weeks, so our analysis assumes that no viral rebounds occurred between scheduled measurements. We could not find a plausible rationale for the detected effect of clinical site on the virological outcome and possible explanations were listed in Chapter 7.5. Moreover, no genotyping was conducted at enrolment, so we were not able to assess the impact of pre-existing NNRTI resistance on response. Lastly, antiretroviral therapy consists of a combination of drugs and its efficacy depends on all the components of the tested regimen and our findings might not be generalizable to efavirenz or nevirapine-based treatment accompanied by drugs different to the ones studied in CHAPAS-3.

Furthermore, in the second study (efavirenz PK/PD – Chapter 5) due to a low frequency of CNS AEs we were not able to confirm their association with high efavirenz exposures. Recent studies in adults indicate that CNS AEs might be underreported and in particular in children more sophisticated methods based on cognitive investigations are required.[250,251]

In addition in the fourth study (nevirapine PK/PD – Chapter 7) most viral loads were matched with nevirapine concentrations measured 12 weeks earlier, and one could argue that drug concentrations measured on the same day as viral load could be more predictive of virological outcome. However, suppression is likely related to maintained drug exposure above a certain threshold, and a random measurement in the time period preceding it could be an adequate indicator of it.

8.6 Conclusions to stated hypothesis

In conclusion SNPs 516G>T and 983T>C in *CYP2B6* are the main predictors of variability in efavirenz and nevirapine concentrations in HIV-infected African children treated with paediatric solid FDC tablets under the WHO simplified weight band dosing, but the variability in drug concentrations is not the sole predictor of treatment outcome. While the genotype guided dose adjustments for efavirenz could significantly contribute towards treatment optimisation by preventing suboptimal drug exposures in extensive metabolisers (leading to increased risk of virological failure) and excessive concentrations in (ultra)slow metabolisers (hypothesised to increased risk of CNS side effects), its application would provide limited benefits for nevirapine. Even though the increase of nevirapine dose in extensive and intermediate metabolisers in the lowest weight band could help prevent sub-optimal drug exposures, the conducted analyses suggest that other interventions could ensure a more beneficial virological outcome. Virological suppression in children treated with nevirapine could be improved if the treatment is started at a lower pre-ART VL and higher pre-ART CD4%. In addition we speculate that virological outcome for both drugs could be improved through interventions enhancing treatment adherence. Based on this reasoning “apriory” 516G>T|983T>C genotype adjusted once daily efavirenz treatment with in combination with abacavir and lamivudine could provide the most optimal virological outcome while minimising CNS side effects in African children >3 years of age. Before the official guidelines can be modified a “point of care” genotype testing system needs to be developed and the suggested “apriori” dose adjustment should be tested in a prospective study. In addition, more research is required to evaluate efficacy and safety of efavirenz treatment in children <3 years old before this approach could be extrapolated to this age group.

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